‘CORRELATION BETWEEN THE SPOT URINE PROTEIN CREATININE RATIO, SPOT URINE ALBUMIN CREATININE RATIO AND 24 HRS URINE PROTEIN ESTIMATION IN PATIENTS WITH NEPHROTIC RANGE PROTEINURIA’

Dissertation on submitted to

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In partial fulfillments of the regulations

for the award of the degree

M.D. BRANCH – I (GENERAL MEDICINE)

GOVT. STANLEY MEDICAL COLLEGE & HOSPITAL,CHENNAI

APRIL 2012

CERTIFICATE

This is to certify that this dissertation entitled "**CORRELATION BETWEEN THE SPOT URINE PROTEIN CREATININE RATIO, SPOT URINE ALBUMIN CREATININE RATIO AND 24 HRS URINE PROTEIN ESTIMATION IN PATIENTS WITH NEPHROTIC RANGE PROTEINURIA**" submitted by **Dr. PATIL DEVENDRA VIJAY** to **The Tamil Nadu Dr. MGR Medical University** is in partial fulfillment of the requirement for the award of **M.D. DEGREE (GENERAL MEDICINE) (BRANCH-I)** and is a bonafide research work carried out by him under direct supervision and guidance.

DR. MAGESHKUMAR S. M.D

Signature of Unit Chief , Professor and HOD

Signature of the Dean

DECLARATION

I solemnly declare that the dissertation entitled "**CORRELATION BETWEEN THE SPOT URINE PROTEIN CREATININE RATIO, SPOT URINE ALBUMIN CREATININE RATIO AND 24 HOURS URINE PROTEIN ESTIMATION IN PATIENTS WITH NEPHROTIC RANGE PROTEINURIA**" was done by me at Government Stanley Medical College and Hospital during 2009-2011 under the guidance and supervision of **PROF. and HOD Dr. S. MAGESHKUMAR M.D**. The dissertation is submitted to the Tamil Nadu Dr. M G R Medical University towards the partial fulfillment of requirements for the award of M.D. DEGREE (BRANCH –I) in General Medicine.

PLACE :

DATE : Dr. Patil Devendra Vijay

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CONTENTS

LIST OF ABBREVATIONS

ACR	Albumin creatinine ratio
PCR	Protein creatinine ratio
HBsAg	Hepatatis B surface antigen
HBeAg	Hepatitis B ‘e’ (pre core) antigen
HIV	Human Immuno deficiency Virus
Ab / Ag	Antibody / Antigen
RPR	Rapid Plasma Reagin
VDRL	Veneral Disease Research Labaratory (Test)
UPEP	Urine Protein electrophoresis
SPEP	Serum protein electrophoresis
ANA	Anti nuclear Antigen
Anti ds DNA	Anti double stranded DNA antibody
U1 RNP	U1 – Ribonucleic protein
NSAID	Non steroidal anti inflammatory drugs
DM	Diabetes Mellitus
FSGS	Focal Segmental Glomerulosclerosis
GFR / eGFR	Glomerular Filteration Rate / estimated Glomerular filteration rate
IHD / CAD	Ischemic heart disease / coronary artery disease

INTRODUCTION

INTRODUCTION

Urine analysis is an important tool in clinical medicine . Proteinuria is a condition in which urine contains an excess amount of proteins. Proteinuria is sometimes the only evidence of severe kidney disease. Detection of proteinuria uncovers renal diseases and also frequently points to a specific diagnosis. Testing the urine for proteinuria has been part of the routine clinical examination. A positive urine protein dipstick test usually initiates the evaluation for proteinuria. Normal daily protein excretion in an adult does not exceed 150 mgs.[2] Persistent proteinuria of >1 gm/day, usually indicates renal disease. Proteinuria may be minimal (<1.0 gm/day), moderate (1–3 gm/day) and heavy (>3 gm/day).A proteinuria greater than 3.5 gm/1.7 m2 body surface area is called nephrotic range proteinuria.[2] Important causes of minimal proteinuria are chronic pyelonephritis, diabetic nephropathy, interstitial nephritis and chronic renal failure. Moderate proteinuria is seen in nephritic syndrome and toxic nephropathies and heavy proteinuria indicates active glomerulonephritis. So quantification of protein is of utmost importance. A 24 hours urine protein estimation is a gold standard technique for the quantitative estimation of proteinuria. However it has few limitations. About 20% of the samples collected are rejected due to inadequate urine collection[1]. A urine PCR (Protein creatinine ratio) and a urine ACR

(Albumin Creatinine ratio) have been found to be a good predictor of protein estimation over 24 hr urine collection in various studies. But a very few studies have compared urine ACR and urine PCR together in patients with nephrotic range.

OBJECTIVE

OBJECTIVE

1. To assess the relationship between 24 hours urine protein estimation and spot urine PCR (Protein Creatinine Ratio)
2. To assess the relationship between 24 hours urine protein estimation and spot urine ACR (Albumin Creatinine Ratio)
3. To assess which amongst the above mentioned(urine PCR and Urine ACR) is a better predictor of the 24 hours urine protein estimation

REVIEW OF LITERATURE

REVIEW OF LITERATURE :

Definition:

Proteinuria is defined as urinary protein excretion of greater than 150 mg per day or greater than 140 mg / m^2 of body surface area in children.

Normal Physiology:

The functional characteristics of the glomerular capillary filter have been extensively studied by the evaluation of the fractional clearance of molecules of different size , shape and charge.The normal glomerular endothelial cells forms a barrier and holds back cells and other particles. They are penetrated by large pores of 100 nm called 'fenestrae' that can easily be traversed by proteins. The glomerular basement traps most large proteins (>150Kda). Foot process of visceral epithelial cells (Podocytes) cover the urinary side of the glomerular basement membrane. They produce a series of narrow channels (Slit diaphragm) to allow passage of small solutes and water. These slit diaphragm bridges the slits between the foot process of the glomerular basement membrane . Negatively charged heparan sulfate proteoglycans cover the visceral epithelial cells[3]. This negative

charge and size selectivity of glomerular basement membrane impedes the passage of anion molecules such as albumin, globulin and large molecular weight protein across the glomerular wall. The filtered smaller proteins are largely reabsorbed. This reabsorbtion takes place in the proximal tubule. Only small amount of the fltered load is excreted. There is also a shape restriction of molecules that allows elongated molecules to cross the glomerular capillary wall more readily than molecules of the same molecular weight.

Multiple factors have been proven to be important in the disruption of the glomerular capillary wall. These include tissue-degrading enzymes, complement components that assemble upon it, get deposited and oxygen radicals that target both the glomerular basement membrane and the slit diaphragm. Heparinase and hyaluronidase alterations in the amino glycan content of the glomerular capillary wall may play a role in increased protein excretion. Exciting clues to the specific components of the glomerular capillary wall, including mutations in the podocyte or proteins in the slit diaphragm, which result in proteinuria are coming in light due to studies based on molecular activity and genetics.[2] Impaired reabsorption of plasma proteins by proximal tubular epithelial cells is also another major mechanism resulting in proteinuria. A number of low-molecular-weight proteins, including β 1, β 2, and α1 microglobulins, are filtered by the glomerulus and absorbed by tubular epithelial cells. When tubular epithelial cells are damaged,

these proteins are excreted. Based on the qualitative nature of proteinuria it is observed that excretion of high-molecular-weight proteins (e.g., fractional excretion of IgG) is indicative of glomerular damage. Similarly, tubular epithelial damage is more likely when there is excretion of low-molecular-weight proteins (e.g., fractional excretion of alpha1 microglobulin. This separation of high- from low-molecular-weight proteinuria has been suggested to be a predictor of clinical outcome in a number of glomerular diseases.A reaction that results in tubular atrophy and interstitial fibrosis has been found to occur as a consequence of the uptake of filtered proteins, including albumin by tubular cells.

Classification of Proteinuria

Table 1 Classification of Proteinuria [2]

PROTEINURIA	
Benign Causes	Pathological Causes
1. Orthostatic /Postural	1. Glomerular
2. Functional	2. Tubular
3. Idiopathic / Intermittent	3. Overflow

BENIGN PROTEINURIA :

It is usually a transient phenomenom. On repeated testing proteinuria disappears. The renal function is normal and there is no significant pedal oedema and blood pressure alterations. The urine sediment is bland and the 24 hours collection is usually less than 1 gm.

1) POSTURAL /ORTHOSTATIC PROTEINURIA

The term "orthostatic proteinuria" is defined by the absence of proteinuria while the patient is in a recumbent posture and its appearance during upright posture, especially during ambulation or exercise. The total amount of protein excretion in a 24-hour period is generally less than 1.0 gram, but may be as much as 2 grams. Orthostatic proteinuria is uncommon in individuals over the age of 30. Orthostatic proteinuria is more common in adolescents. Two to five percent of adolescents have orthostatic proteinuria. It is diagnosed by split urine protein excretion examination.[4] In orthostatic proteinuria, the day time specimen typically has an increased concentration of protein, with night time specimen having a normal concentration usually less than 50 mg over eight hours. In true glomerular disease there is reduced protein excretion in the supine position but it will not return to normal as with orthostatic proteinuria. . Little convincing data exists on the usefulness of urinary protein-to-creatinine ratio measurements during recumbency versus ambulation as a diagnostic test for orthostatic proteinuria.

Data on renal biopsies on orthostatic proteinuria are confusing. Some showed minor glomerular changes. Springberg et al.[5] found that long term prognosis of orthostatic proteinuria is benign in virtually all cases over many decades. An explaination given for postural proteinuria is that posture affects urinary protein excretion, probably via an increase in glomerular capillary hydrostatic pressure and change in permeability of the glomerular capillary walls. An alternate explanation given suggests possible entrapment of renal veins as a cause of proteinuria.

2). FUNCTIONAL PROTEINURIA

It is seen during febrile illness, heavy physical exertion,emotional stress and cardiac failure. It is usually less than 0.5 gm/day but may be as heavy in few cases. It disappears with the resolution of causative disorder . Poortmans et al[6] and Kallmeyer et al[7] found that several gram of protein per liter of urine together with haematuria and even casts can be occasionally seen after exercise, especially jogging (jogger's nephritis)[2]. Post exercise proteinuria is about 15 to 20 times the resting range of proteinuria. Resolution to normal range protein excretion may require about 4 hours . they also found that proteinuria was influenced mostly by the intensity of exercise rather than its duration.

3) IDIOPATHIC PROTEINURIA

This is seen in young healthy adults. This dipstick positive proteinuria disappears spontaneously by next clinical visit.

PATHOLOGICAL PROTEINURIA

1) GLOMERULAR PROTEINURIA

A number of factors have proven to be important in the disruption of the glomerular capillary wall . These include tissue-degrading enzymes, immune complexes and complement components that assemble upon it, oxygen radicals that target both the glomerular basement membrane and the slit diaphragm, loss of the fixed anionic charge , disruption of the barrier, detachment of the epithelial podocytes. Heparinase and hyaluronidase alterations in the amino glycan content of the glomerular capillary wall may play a role in increased protein excretion. It is very common in clinical practice.Albumin is the major protein excreted in glomerular proteinuria and comprises of 85 – 90% of total protein excreted. Other proteins include relatively low molecular weight proteins like pre-albumin, orosomucoid, transferrin. McConnell et al on evaluation of proteinuria found that urinary excretion of more than 2 gm per 24 hours is usually a result of glomerular disease .

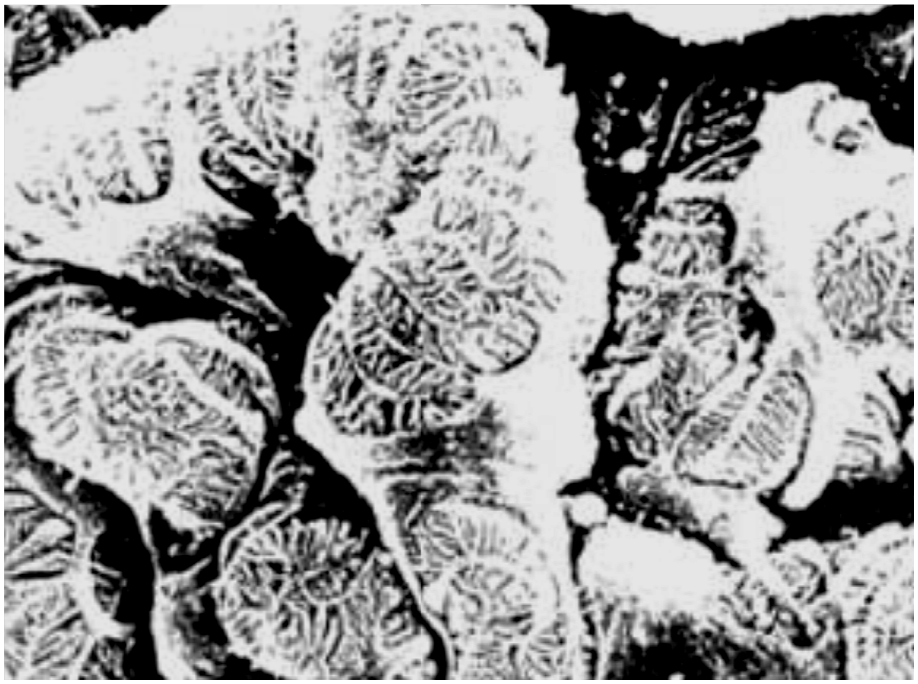


Figure 1:Scanning electron microscopy of the glomerulus. The surface anatomy of the interdigitating foot processes of normal visceralepithelial cells (podocytes) is demonstrated. These cells and their processes cover the capillary, and ultrafiltration occurs betweethe fine branches of the cells.

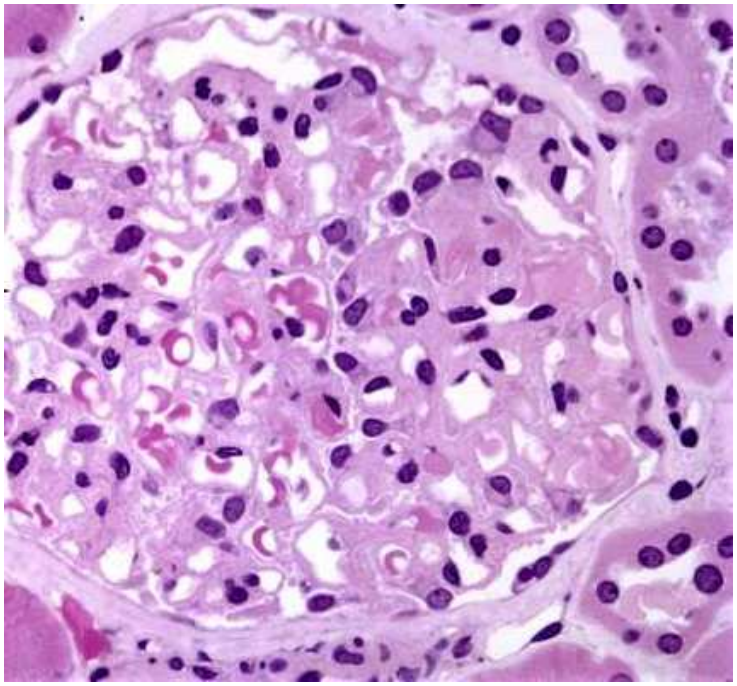


Figure 2: Membranous Nephropathy: The capillary loops are thickened and there is expansion of the mesangial regions by the deposition of matrix. When a immunoflorescence is done it shows granular subepithelial IgG along the basement membrane.

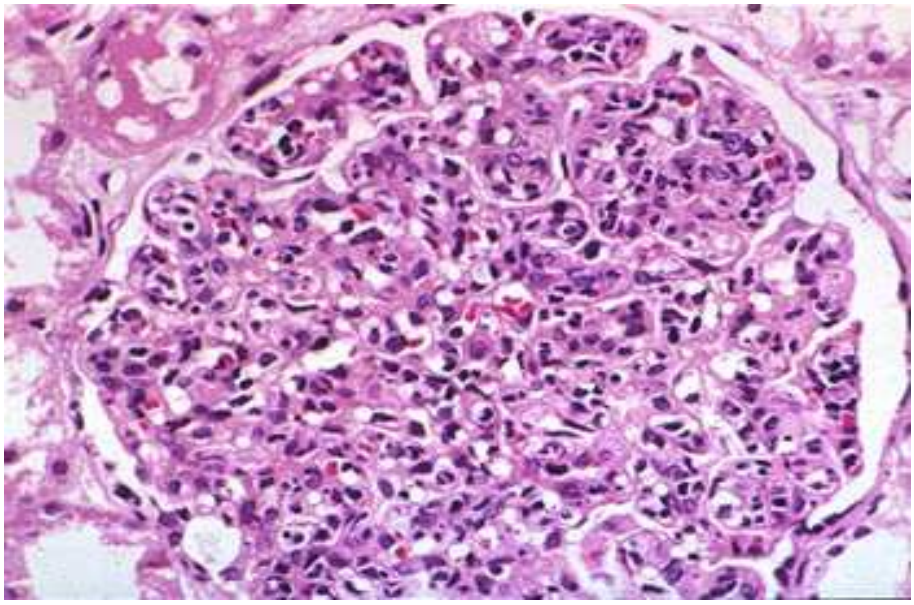


Figure 3: Lupus nephritis DPGN. This image shows the diffuse endocapillary proliferative pattern of mesangial cells with infux of moncytes and granulocytes.

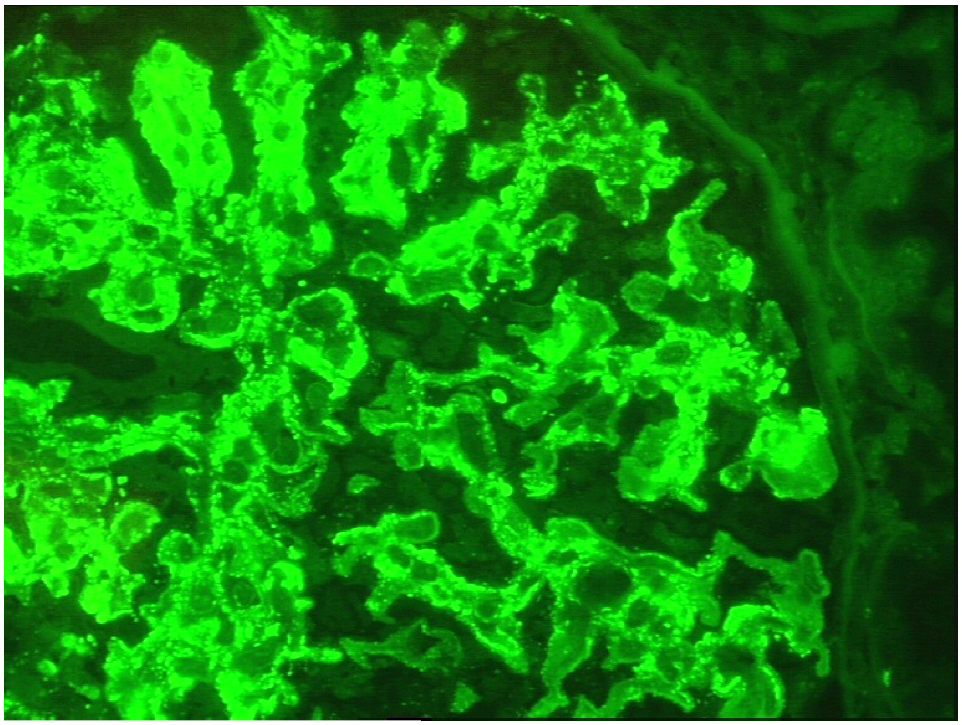


Figure 4: Full house in SLE. This image shows florid immune florescent deposition of IgG , IgM , IgA , C3 and C1q in the glomerulus –a pattern that is referred as 'Full-House' seen especially in SLE Nephritis.

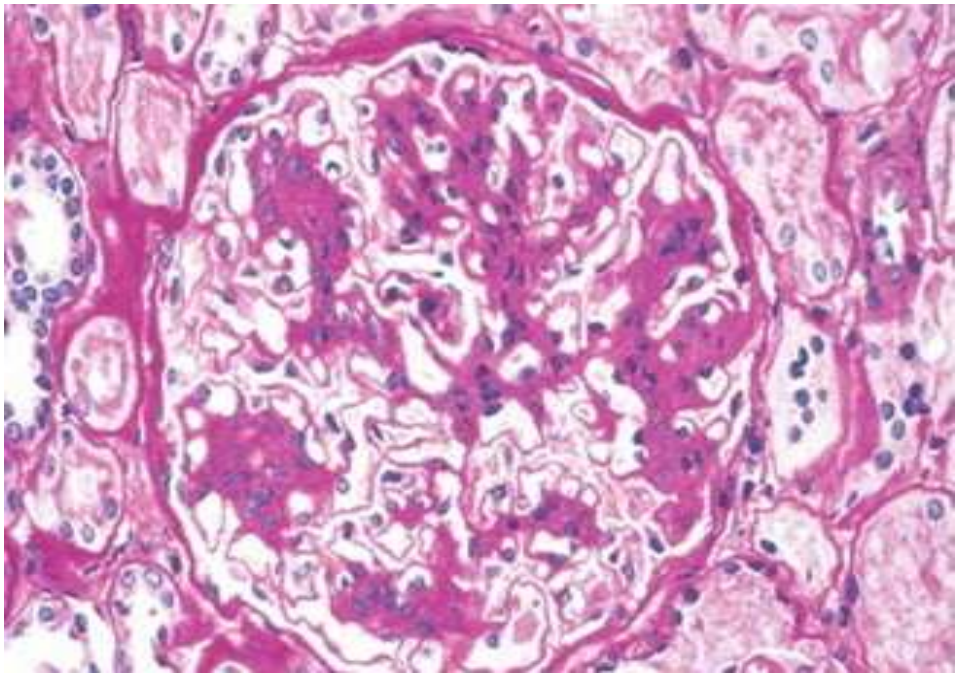


Figure 5: IgA disease. This light microscopy shows widening with an increase in cellularity in the mesangial regions and this process has affected the lobules of some glomeruli to a greater degree than others.

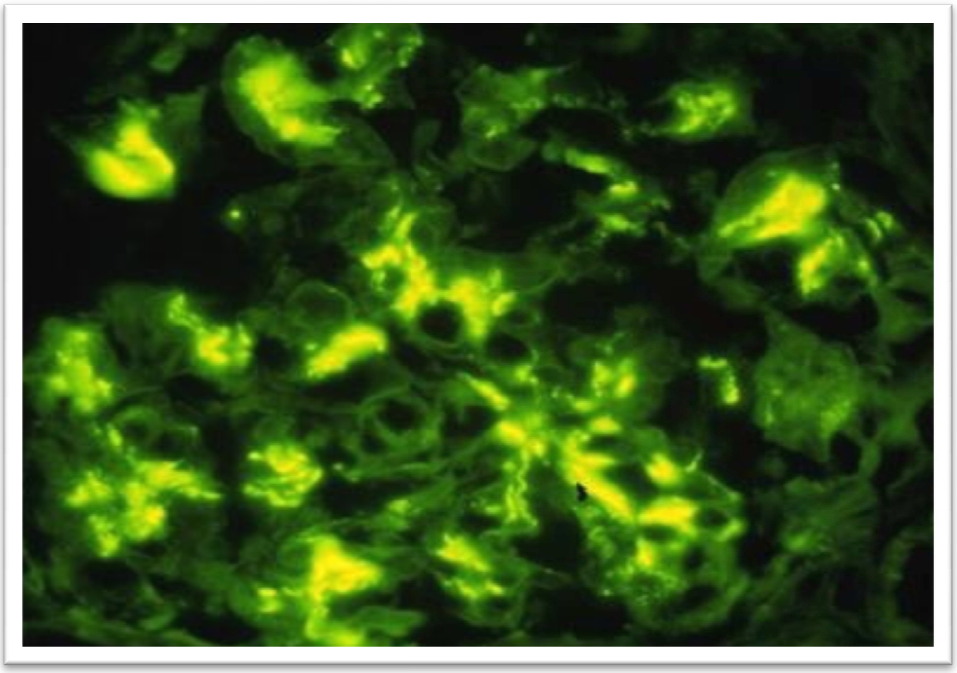


Figure 6: Immumoflorescence microscopy image in IGA nephropathy : Granular mesangial deposits of IgA are seen with associated complement C3, and IgG or IgM, or both. IgG and IgM often are seen in lesser degrees of intensity than is IgA.

Table 2. Systemic Diseases that Cause Glomerular Injury and a Nephrotic Clinical Presentation[8]

Disease state	Common stiologies	Laboratory findings
Infections	Hepatitis B (C less common)	HBsAg, HBeAg
	HIV	HIV Ab
	Syphilis	RPR, VDRL
Chronic diseases	Diabetes	ElevatedHbA1c, sugars
	Amyloidosis	UPEP/IEP
	Sickle cell disease	Hemoglobin electrophoresis
	Obesity	
Malignancies	Multiple myeloma	SPEP, UPEP
	Adenocarcinoma (lung, breast, colon most common) , Lymphoma	
Rheumatologic	Systemic lupus erythematosus	ANA, anti-dsDNA Ab
	Rheumatoid arthritis	Rheumatoid factor
	Mixed connective tissue disease	Anti-U1-RNP Ab
Medications	NSAIDs , Penicillamine, Captopril, Gold,etc.,	

(refer to page for abbreviations used.)

Common Glomerular injury patterns [4]:

- Minimal change disease
- Focal segmental glomerulonephritis
- Idiopathic membranous glomerulonephritis
- Membranoproliferative glomerulonephritis
- IgA nephropathy

2) TUBULAR PROTEINURIA

Failure to reabsorb small-molecular-weight proteins is caused by damage to the renal tubulointerstitial region. This leads to tubular proteinuria. The most prevalent tubular protein (and the most abundant protein in normal urine) is Tamm-Horsfall protein, which enters the urine after synthesis in the tubular cells of the ascending limb of the loop of Henle and is secreted into the urine[3]. Under normal conditions, the small amount of urinary protein is composed of filtered proteins from plasma (50%) and proteins that are secreted into the urine from urinary tract cells (50%). Filtered proteins include small amounts of albumin (approximately 15% of the total urinary protein), immunoglobulins (5%), light chains (5%), β2-microglobulin (<0.2%), and other plasma proteins (25%).[2] Under conditions of tubulointerstitial injury, both filtered and secreted proteins are found in increased amounts in the urine, up to 1 to 2 g per day. Multiple

mechanisms are responsible for tubular proteinuria. Injured tubules are unable to reabsorb the small-molecular-weight proteins normally filtered by the glomerulus, such as β2-microglobulin. Also as a result of tubular injury brush border components and cellular enzymes such as n-acetylglucosamine and lysozyme are secreted into the urine. Lastly, increased amounts of Tamm-Horsfall protein may be secreted into the urine by injured tubular cells of the ascending limb of the loop of Henle and the distal nephron.

Table 3. TUBULAR PROTEINURIA – common causes[9]

Hypertensive nephrosclerosis	Acute hypersensitivity
Polycystic Kidney disease	Interstitial nephritis
Pyelonephritis	Oxalosis
Obstruction	Cystinosis
Vesico – ureteric reflux	Hypercalcemia
Fanconi syndrome	Hyperuricemia
Heavy metals	Sickle cell disease
Uric acid nephropathy	Drugs (NSAID, Antibiotics)

3) OVERFLOW PROTEINURIA

It is due to filtration by normal glomerulus of an abnormally large amount of low molecular weight proteins, which exceeds the capacity of the normal tubules for reabsorption. It is characterized by the presence of abnormal peak or spike on urinary electrophoresis. Most often, this is a result of the immunoglobulin over production that occurs in multiple myeloma. The resultant light change immunoglobulin fragments (Bence Jones proteins) produce a monoclonal spike in the urine electrophresis.

OVERFLOW PROTEINURIA –CAUSES [9]

-Multiple myeloma

-Myoglobinuria

-Rhabdomyolysis

-Lymphoproliferative disorders

SELECTIVITY OF PROTEINURIA :

The type of protein excreted gives a clue to the likely origin of the proteinuria and the likely pathology. The type of protein excreted can be ascertained by various methods like urine protein electrophoresis , immune electrophoresis . In glomerular proteinuria, a urine protein electrophoresis (UPEP)

demonstrates primarily albumin rather than globulins, whereas tubular proteinuria demonstrates a predominance of small-molecular-weight proteins. (Immune electro phoresis) IEP can quantify this distinction further if a definitive spike is not present on UPEP. A urinary albumin to β2 microglobulin ratio of 10 to 1 is indicative of tubular proteinuria, in contrast to glomerular proteinuria, in which this ratio usually exceeds 1000:1. In comparison, in normal urine, the albumin to β2 microglobulin ratio ranges from 50:1 to 200:1. Evaluation of overflow proteinuria may be aided by UPEP, which separates urinary proteins into five peaks based on the molecular weights of the proteins. The five peaks include albumin and α_1, α_2, β_2, and gamma globulins. For example, an abnormal peak or spike occurring in the gamma region suggests the presence of a monoclonal gammopathy. A selectivity index is calculated by the ratio of IgG clearance to the albumin clearance[13]. If the selectivity index is lesser then 0.1 , then the proteinuria is highly selective and if it is greater than 0.2 then its non selective.

$$Selective\ Index = \frac{IgG\ Urine}{IgG\ Serum} \times \frac{Serum\ Albumin}{Urine\ Albumin}$$

According to Tay et. al[12] another formula can also be used for the calculation of selectivity index of proteinuria.

$$Selective\ Index = \frac{IgG\ Urine}{IgG\ Serum} \times \frac{Serum\ Transferrin}{Urine\ Transferrin}$$

A ratio of <0.16 indicates highly selective proteinuria. In children, minimal change nephropathy causes selective proteinuria, whereas non-selective proteinuria raises the possibility of an alternative type of renal disease and might lead to a recommendation of renal biopsy to avoid steroid treatment when this would be unlikely to be of benefit. Measurement of selectivity in adults is of very limited use.

MICROALBUMINURIA :

Microalbuminuria is defined as 30 to 300 mg/d in a 24-h collection or 30 to 300 microgm/mg creatinine in a spot collection (preferred method). The appearance of microalbuminuria (incipient nephropathy) in DM is an important predictor of progression to overt proteinuria (greater 300 mg/d) or overt nephropathy and is an independent risk factor for cardiovascular morbidity. Besides diabetes mellitus, a number of other conditions have been found to be associated with microalbuminuria, including female gender, old age, etc. (Jones et al. 2002)[18].

Albuminuria was also found in high blood pressure patients (Rosa and Palatini 2000)[14].Accordingly Pannacciulli et al. 2001[15], found that obesity and hyper-triglyceridaemia as well as smoking (Gambaro et al. 2001)[16] was

associated with albuminuria. Oral contraceptive use and hormone replacement therapy (Monster et al. 2001)[17] has been shown to increase albuminuria.

METHODS OF DETECTING AND MEASURING PROTEINURIA

A) DETECTION OF PROTEINURIA

1. Dipstick analysis
2. Precipitation methods

B) QUANTIFICATION OF PROTEINURIA

1. Turbidimetric method
2. Biuret method
3. Dye binding technique

C) CHARECTERIZATION OF PROTEINURIA

1. Immune electrophoresis
2. Column gel chromatography
3. Agarose gel / Polyacrylamide gel electrophoresis

1) DIPSTICK ANALYSIS

It is used in most out-patient settings to detect proteinuria. It semi quantitatively measures the urine protein concentration. Paper strip is impregnated with indicator dye like bromocresol green. It changes colour in presence of protein. In the absence of protein the dipstick panel is yellow. With increasing

concentrations of protein in urine the dye indicators undergo sequential colour changes from pale green to green and blue. The binding of a protein to the indicators, which are structurally similar to bromocresol green, is highly pH dependent. Albumin binds to indicators at pH between 5 and 7. Other proteins bind at lower pH, but with a lower affinity than albumin, while Bence Jones protein does not bind at any pH. Hence , it preferentially detects negatively charged urinary proteins like albumin. However albumin levels between 30-300mg/dl are not detected. Light chains and some low molecular weight protein are not detected by stick tests. The sticks are buffered to keep the pH constant. Leaving the sticks in the urine will wash out the buffer and give a false reading. They should be read immediately. Sticks are very sensitive giving a trace or positive reading with many normal urine samples containing only about 100 mg/l of protein The results are expressed on a scale from 0 to +++ or ++++, at each of which correspond approximate protein concentrations, which vary according to the manufacturer.

FALSE POSITIVE DIPSTICK PROTEINURIA

- (alkaline urine) urine pH > 7
- Highly concentrated urine
- deeply pigmented urine
- quaternary ammonium compounds

- phenazopyridine

- Gross Haematuria

- Dipstick immersed too long

- Presence of Penicillin, Sulfonamide or Tolbutamide.

- Pus

- Semen /vaginal secretions

FALSE NEGATIVE DIPSTICK PROTEINURIA

- Dilute urine (specific gravity > 1.015)

- When the urinary proteins are non albumin

Especially in case on the low molecular weight proteins.

- Bence Jones proteins

QUANTITATIVE ANALYSIS OF PROTEINURIA

Table 4. Tests for Quantitative detection of Proteinuria [2]

TEST	Analytical sensitivity (mg/l)	Linearity (mg/l)	Distinctive features
Turbidimetric			
Sulfosalicylic acid	10-20	10-3000	Albumin overestimation; some glycoproteins are not detected;
Trichloroacetic	20	20-2400	Same sensitivity for albumin and globulins;many drugs can interfere;
Benzethonium chloride	10	10-1600	Albumin over-estimation; under-estimation of increased protein concentrations;less turbidity for gamma globulins than for albumin;
Dye binding			
Coomassie brilliant blue	2.5	5-1500	High sensitivity; underestimation of tubular proteins; interference from various metabolites, drugs.
Poncea	20	100-1600	Same sensitivity for albumin and globulins; positive interference with aminoglycoside antibiotics;
Biuret(Precipitation)			
Tsuchiya reagent		5-2000 (volume – 2ml)	very few interferences **reference method recommended by the American Association for Clinical Chemistry**
Folin-Lowry reagent	10	10-700	Interference by tyrosine

QUALITATIVE ANALYSIS

Several methods are available for the qualitative analysis of proteinuria. Electrophoresis Electrophoresis on cellulose acetate or agarose after protein concentration or using very sensitive staining (silver or gold stains) is one of the most widely used method. Better resolution is obtained by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), which detects urinary proteins on the basis of their molecular weight. This technique allows the identification of proteins of tubular origin with low molecular weight (e.g. 10 kDa)

SAMPLE COLLECTION:

The method of collecting a urine sample is of critical importance especially when the specimen is to be examined microscopically. As suggested by (Kouri et al. 2000)[19]. It is preferable to give written instructions describing the procedure to the patient to avoid errors in sample collection. These should include avoiding strenuous physical exercise (e.g. marathon, jogging) in the hours before the collection period. This is because such activities may lead to physiologic proteinuria and/or haematuria. Collection during menstruation is alsoavoided as

there is a risk of contamination with blood. Depending on the clinical diagnosis and suspicion, a timed urine collection can be requested. For e.g. 24 hr collection , overnight collection or a spot urine collection may be needed.

A 24 hr urine collection involves starting the collection time in morning by emptying the bladder and discarding the first morning urine, then collecting all urine for the subsequent 24 hours, including the first morning void the following day. The urine should be preferably refrigerated during the entrie collection period. This is not possible in many situations and so an alternative method is advocated by adding one cup of vinegar to the collection container to act as a preservative.

In case, a spot sample is requested at least 50 ml of urine should be collected. Urine should be collected in a container supplied by the laboratory. It should have a capacity of at least 50-100 ml and a diameter opening of at least 5 cm to allow easy collection by both females and males (Kouri et al. 2000)[19].

STORAGE OF SPECIMENS :

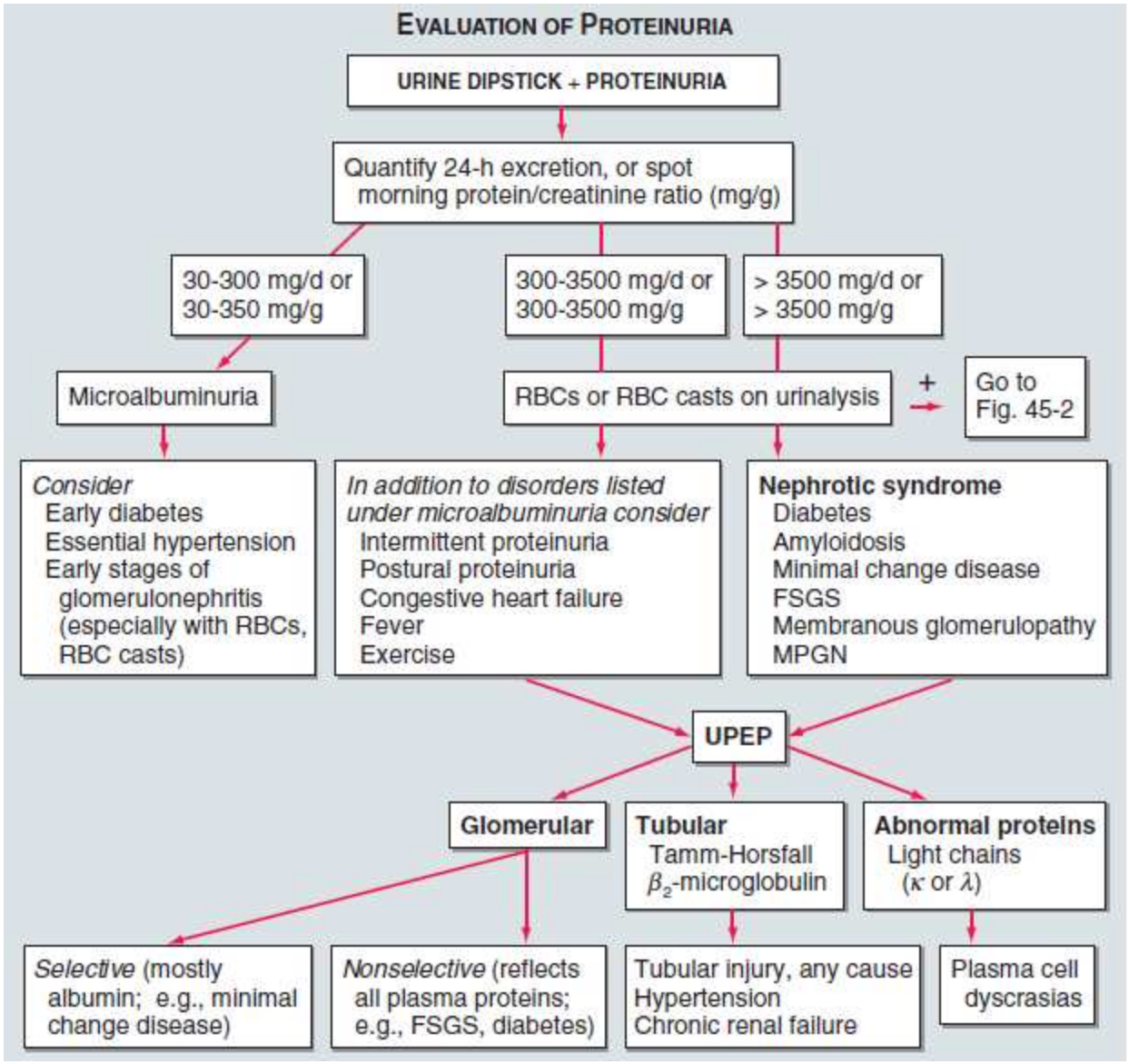
Analysis within one hour of voiding is done to avoid alterations in physical or chemical features. Several means of preservation, such as addition of thymol, borate or toluene or refrigeration at 4°C have been proposed. Some of these can,

however, cause some interfering chemical reactions. For e.g. formalin may in some cases precipitate protein and thymol does interfere with the acid precipitation test for proteins. Thus, there is no ideal preservative yet and hence the study of fresh urine is always advised.

ADEQUACY OF SAMPLE COLLECTION :

To ensure adequate collection, a 24-hour total creatinine excretion should be obtained on the same sample. In females under steady-state conditions of renal function, the 24-hour urinary excretion of creatinine should equal approximately 15 to 20 mg per kg of ideal body weight; in males, the excretion should be 18 to 25 mg per kg of ideal body weight. Creatinine is produced at constant rate and in an amount directly proportional to skeletal muscle mass. With steady state day-to-day renal function, each gram of Creatinine in 24 hour urine collection represents 18.5 gms of fat free skeletal muscle[20]. Since concentration of Creatinine remains relatively constant on a daily basis, in patients with a steady state of renal function, it can be used to assess the adequacy of timed urine collections.

Figure 1: APPROACH TO A PATIENT WITH PROTEINURIA [21]



METHODOLOGY

METHODOLOGY:

Source of Data:

A total of 72 patients attending the department of Internal Medicine and department of Nephrology of Government Stanley Medical College, Chennai , on both out-patient basis and in-patient basis were included in this study.

Duration of Study : 1 year (July 2010 – June 2011)

INCLUSION CRITERIA

1) Age < 80 years
2) Patients with proteinuria > 3.5 gms /24 hours
3) Patients on immunosuppressive therapy for Glomerulo-nephritis
4) Patients of either sex
5) Patients not dependent on Hemo-dialysis

EXCLUSION CRITERIA

1) Patients of age less than 14 years
2) Gross Haematuria
3) Patients with febrile illness

4) Inadequate sample collection (An inadequate urine sample was defined as calculated 24 hours urine creatinine excretion out of range to the expected total 24 hours urine creatinine(15-20mg/ideal body weight for females and 18-25 mg/kg ideal body weight for males)
5) Heart Failure
6) Patients on Anti-Proteinuric drugs (Eg: ACE inhibitors ,Angiotensin receptor Blockers, sulphonamides)
7) Head Injury
8) Intense physical exertion
9) Dehydration
10) Patients with urine output less than 400 ml per 24 hours.

METHOD OF DATA COLLECTION:

All patients attending the General Medicine and Nephrology Out Patient / In Patient Department and having significant proteinuria were asked to collect 24 hours urine protein. Instructions were given to the patient. Then they were asked to void the first morning sample and then collect urine from that day onwards till the next day including the morning first void sample. Urine was collected in a 5 litre sterile plastic can with a 25 ml of acetic acid or 5-10 ml of conc. Hydrochloric

acid, added as preservative. The collected sample was analysed for 24 urine protein estimation using the turbidimetry method using sulfosalicyclic acid.

Patients who had a 24 hours urine protein excretion more than 3.5 gm and satisfying the mentioned inclusion and exclusion criteria were identified. They were given an informed consent form and only after the receipt of their signatures/ thumb print on the informed consent form, they were enrolled into the study. Blood samples were also collected and sent for analysis. Patients were also requested to collect their first void sample the next morning and this sample was analysed for urine PCR and urine ACR. The PCR was calculated using the following formula:

$$\text{PCR} = \frac{\text{Quantitative estimation of protein using the sulfosalicyclic acid turbidimetry method (in mg\%)}}{\text{Quantitative creatinine estimation using modified Jaffe's method(in mg\%)}}$$

The ACR was calculated using the following formula:

$$\text{ACR} = \frac{\text{Quantitative estimation of albumin using the immuno turbidimetry method (in mg\%)}}{\text{Quantitative creatinine estimation using modified Jaffe's method(in mg\%)}}$$

Then again the same day a 24 hours urine protein sample was collected as per the above mentioned method.

GFR was calculated based on Cockcroft-Gault equation.

Estimated creatinine clearance (mL/min) = [(140 – age) × body weight(kg)] /

[72 × serum Creatinine (mg/dL)]

The product was multiplied by 0.85 if the patient is female.

The patients were divided into groups depending on their eGFR.

1). Patients with eGFR > 30 ml /min

2). Patients with eGFR < 30 ml/min

3). Patients with GFR < 15ml/min.

The correlation coefficient (r) was computed using the following formula:[78]

r = Correl(x,y) where 'x' and 'y' are the variables and x- any y- are their respective mean values.

$$Correl(X,Y) = \frac{\sum (x-\bar{x})(y-\bar{y})}{\sqrt{\sum (x-\bar{x})^2 \sum (y-\bar{y})^2}}$$

The same formula was used for calculation of coefficient of correlation

- for the entire sample population (24 hours urine protein and PCR)

-- for the entire sample population (24 hours urine protein and ACR)

- then separately for Group A and B

- for patients with Stage 5 CKD

- for the entire sample population (24 hours urine protein and PCR) in logarithmic.

Finally the strength of correlation was compared.

RESULTS

RESULTS:

This study included 72 patients, who had 24 hours urine protein of >3.5 gms/day with varying degree of renal dysfunction. The patients were segregated into groups based on their estimated creatinine clearance rate and the data was tabulated and analysed.

Age Distribution in study population :

Table 5. Age Distribution

Age Group	No. of patients	Percentage
14 – 30 years	27	38
31 – 60 years	37	51
61 years and above	8	11

In this study , the age of patient ranged from 14 to 78 years. The incidence of nephrotic range proteinuria was maximum around 31 to 60 years(51%).

Table 7: Age Distribution

Age distribution of patients	Years
Range	14 – 78
Mean	37.89
Median	35

Table 7: Sex distribution in the study population :

Sex of the patients	No. of patients
Male	40
Female	32

In this study there were 40 men (56%) and 32women(44%).

Medical Illness in Patients with Nephrotic range proteinuria

Table No. **8. Co-morbid Medical Illness in Patients with Nephrotic range Proteinuria**

Risk Factor	No. of patients	Percentage
Hypertension	44	61
DM	0	0
SLE	7	10
Hepatitis B	3	4
Hepatitis C	1	1
HIV	1	1
NSAID use	27	38
Family History	0	0
Drugs / Native medicines	7	10
IHD / CAD	0	0
Hypothyroidism	4	6
None	15	21

Because few patients had multiple risk factors the total of percentage coloumn will not be 100.

Accordingly , it was found that there is a maximum incidence of Hypertension in patients with nephrotic range proteinuria (61%). In this study, the

patients having Diabetes mellitus were not included as almost all of them were on Tab. Enalapril which acts as a anti-protenuric drug and hence may act as a confounding factor. NSAID use was present in 38% patients and it was prevalent in age group >30 years. There were total of 7 patients(10%) who were suffering from lupus nephritis. There were 21% patients who did not harbor any of the mentioned risk factors and co-morbid illness.

Etiology based on Biopsy Results:

Table 9. Etiology based on Biopsy Results:

HISTOLOGICAL DIAGNOSIS	No. Of Patients	Percentage (%)
Chronic Glomerular Sclerosis	6	8.33
Diffuse Proliferative Glomerulonephritis	9	12.5
Focal Segmental Glomerulosclerosis	11	15.3
IgA Nephropathy	16	22.2
Membranous Nephropathy	14	19.4
Membrano Proliferative Glomerulopathy	4	5.56
Myeloma Kidney	2	2.78
Cresentic Glomerulo-sclerosis	4	5.56
Biopsy Not Done	6	8.33

This table shows the histopathological diagnosis of the patients included in study. Accordingly,it was observed that the most common cause of nephrotic range proteinuria was Ig A Nephropathy followed by Membranous Glomerulo -

Nephropathy. Biopsy was not done in 6 patients. It was interesting to note that 2 patients finally were diagnosed as Myeloma.

The three main renal biopsy diagnosis for patients with nephrotic range proteinuria are IgA nephropathy , Membranous Nephropathy and FSGS. A comparison between the 3 groups was done.

Table 5 : Nephrotic Syndrome Characterstics :

Renal Biopsy Diagnosis	Mean Age (years)	Male: Female Ratio	Urine PCR(mean)	24 hours urine Protein(mean)
Ig A Nephropathy	32.1	9:7	5.67	5.7
Membranous Nephropathy	34.7	1:1	8.93	9.38
FSGS	36.6	8:3	4.97	5.74

According to this table, all the three important causes for nephrotic range proteinuria had a mean age around 32-36 years. There was a male preponderance in Ig A nephropathy and FSGS . However the ratio was 1:1 in Membranous nephropathy. The mean 24 hours urine protein and urine PCR was higher in membranous nephropathy as compared to both FSGS and Ig A nephropathy.

In this study, a total of 7 patients had SLE who had a nephrotic flare. All of them were females with a mean age of 26.1years. The mean 24 hours urine protein

and Urine PCR were 6.37 and 6.59 respectively. The renal biopsy of these patients showed Membranous nephropathy in 4 patients (57%) and DPGN in 2 patients (29%). Biopsy was not done in 2 patients.

Classification of patients based on CKD stage

Table 6. Classification of patients based on CKD stage

CKD Stage	eGFR (in ml/min)	No. of male patients	No. of Female patients	Total No. of patients
I	>90	11	1	12
II	60-89	14	8	22
III	30-59	9	8	17
IV	15-29	4	13	17
V	<15	2	2	4

In this study Stage II CKD occupied the maximum no. of patients - 22 (30%) followed by stage III CKD -17 patients (24%). About 30 % of patients had eGFR below 30 ml/min and this included a total of 4 patients (5%) with stage V CKD. About 47% of patients were having a eGFR 60ml/min and above.

Statistical Analysis :

Table 7:Paired Samples Statistics (24 hours urine protein and Urine PCR)

	Mean	Number	Standard Deviation	Standard Error Mean	Significance 'p' value
24 Hours Urine Protein	6.5739	72	2.1333	0.2514	<0.001
Urine PCR	5.3986	72	2.0198	0.238	

Table 8. Paired Samples Correlations (24 hours urine protein and Urine PCR)

	Number	Co-efficient of correlation (r)
24 Hours Urine Protein and urine PCR	72	0.825

According to this scatter plot , the relationship between 24 hours urine protein and urine PCR is linear (R^2 = 0.561). the graph also shows that urine PCR is almost numerically equal to 24 hours urine protein upto excretion rate of 5 gm/24 hr. however the relationship weakens as the proteinuria increases .

In this study the equation obtained for the calculation of 24 hours urine protein is as follows:

24 hours urine protein = 0.653(urine PCR) + 2.459

Table 9. Paired Samples Statistics (24 hours urine protein and Urine ACR)

	Mean	Number	Standard Deviation	Standard Error of Mean
Urine ACR	4.4684	72	1.82494	.21507
24 Hours Urine Protein	6.5739	72	2.13329	.25141

Table 10. Paired Samples Correlations (24 hours urine protein and Urine ACR)

	Number	Co-efficient of correlation (r)
24 Hours Urine Protein and urine ACR	72	0.636

This is a scatter plot where in the 24 hours urine protein has been plotted against urine ACR of each patient .According to this graph there is a linear regression (R2 = 0.396) . there is considerable variation from the linearity and it widens as we move to right half of the scatter plot. According to this study

24 hours urine protein = 0.729(urine ACR) + 3.309 ($R^2 = 0.396$) or

24 hours urine protein = $3.136[(\text{urine ACR})^{1/2}] + 0.068$ ($R^2 = 0.381$)(using mathematical transformation)

LOGARITHMIC RELATIONSHIP:

In this study , when a scatter plot of log (24 hours urine protein) and log (urine PCR) was plot the relationship obtained had a coefficient of correlation(r) of 0.776 . The equation obtained

Log (24 hours urine protein) = 0.948(log urine PCR) + 0.027 (with $R^2 = 0.6$).

COEFFIECIENT OF CORRELATION:

Table 13. Distance Matrix between urine ACR , PCR and 24 hrs urine protein.

Distance Matrix

	Euclidean Distance		
	ACR	PCR	24 Hours Urine Protein
ACR	.000	21.561	**22.984**
PCR	21.561	.000	**14.008**
24 Hours Urine Protein	22.984	14.008	.000

This is a dissimilarity matrix

According to this table, it clearly shows that when a distance matrix is used to compare the coefficient of correlation between urine PCR and urine ACR with 24 hours urine protein each , urine PCR (Eucliedean distance 14.008) is more closely associated to 24 hours protein than urine ACR(Eucliedean distance 22.984)

Table 11**. Comparison of coefficient of correlation(urine PCR and 24 hours urine protein)**

Group (CKD Stage)	No. of Patients	Correlation (r value)	p value
1 to 3	51 (71%)	0.805	0
4 and 5	21 (29%)	0.724	0
5 alone	4 (5%)	0.682	0

This table shows that as the GFR reduces or as the CKD stage worsens the co-efficient of correlation becomes weaker. It is maximum in patients with GFR > 30 ml/min. (CKD stage 1,2,3) (r = 0.805). however it is weakest at stage 5 CKD disease(r = 0.682).

Table 14: Distance Matrix between urine PCR and 24 hrs urine protein at different stages of CKD.

Distance Matrix

	Euclidean Distance					
	PCR CKD (1to 3)	24 Hrs Urine Protein CKD (1to3)	PCR CKD (4 and 5)	24 Hrs Urine Protein CKD (4 and 5)	PCR CKD 5	24 Hrs Urine Protein CKD 5
PCR CKD (1to 3)	.000	**2.025**	6.273	6.064	7.632	5.629
24 Hrs Urine Protein CKD (1to3)	2.025	.000	5.980	5.714	6.777	4.762
PCR CKD (4 and 5)	6.273	5.980	.000	**3.518**	8.619	8.466
24 Hrs Urine Protein CKD (4 and 5)	6.064	5.714	3.518	.000	8.099	8.782
PCR CKD 5	7.632	6.777	8.619	8.099	.000	**4.086**
24 Hrs Urine Protein CKD 5	5.629	4.762	8.466	8.782	4.086	.000

This is a dissimilarity matrix

According to this table, we plot a distance matrix of urine PCR and 24 hours urine protein at different stages of CKD. This table shows that the Euclidean distance increases as the CKD stage worsens. It is 2.025 in patients with GFR > 30ml/min (CKD stage 1,2 or 3) ,3.518 in patients with GFR < 30 ml/min(CKD stage 4 and 5) and it is 4.086 (maximum) with GFR < 15ml/min (CKD stage 5).

Table 12. **Comparison of coefficient of correlation**

Parameters compared	Co-efficient of co-relation (r)
Urine PCR and 24 hours urine protein	0.825
Logarithmic correlation between urine PCR and 24 hours urine protein	0.776
Urine ACR and 24 hours urine protein	0.636
Urine PCR and 24 hours urine protein (in patients with GFR > 30 ml/min)	0.805
Urine PCR and 24 hours urine protein (in patients with GFR < 30 ml/min)	0.724
Urine PCR and 24 hours urine protein (in patients with GFR < 15 ml/min	0.682
Urine ACR and 24 hours urine protein(in patients with GFR >30 ml/min)	0.672
Urine ACR and 24 hours urine protein(in patients with GFR < 30 ml/min)	0.503

This table shows that the co-efficient of correlation is maximm with 24 hours urine protein and urine PCR.

Figure 1: Age distribution

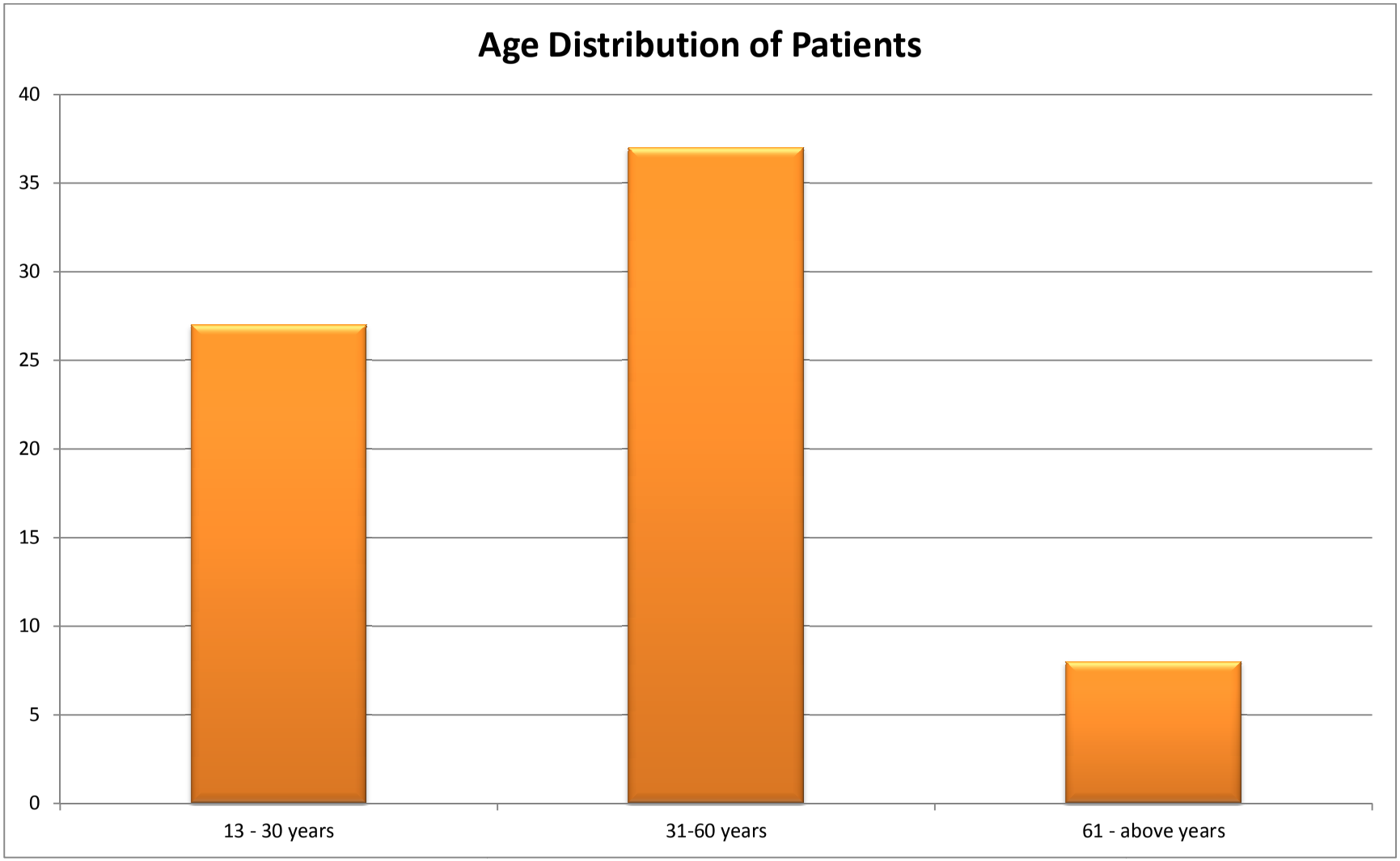


Figure 2: Age distribution:

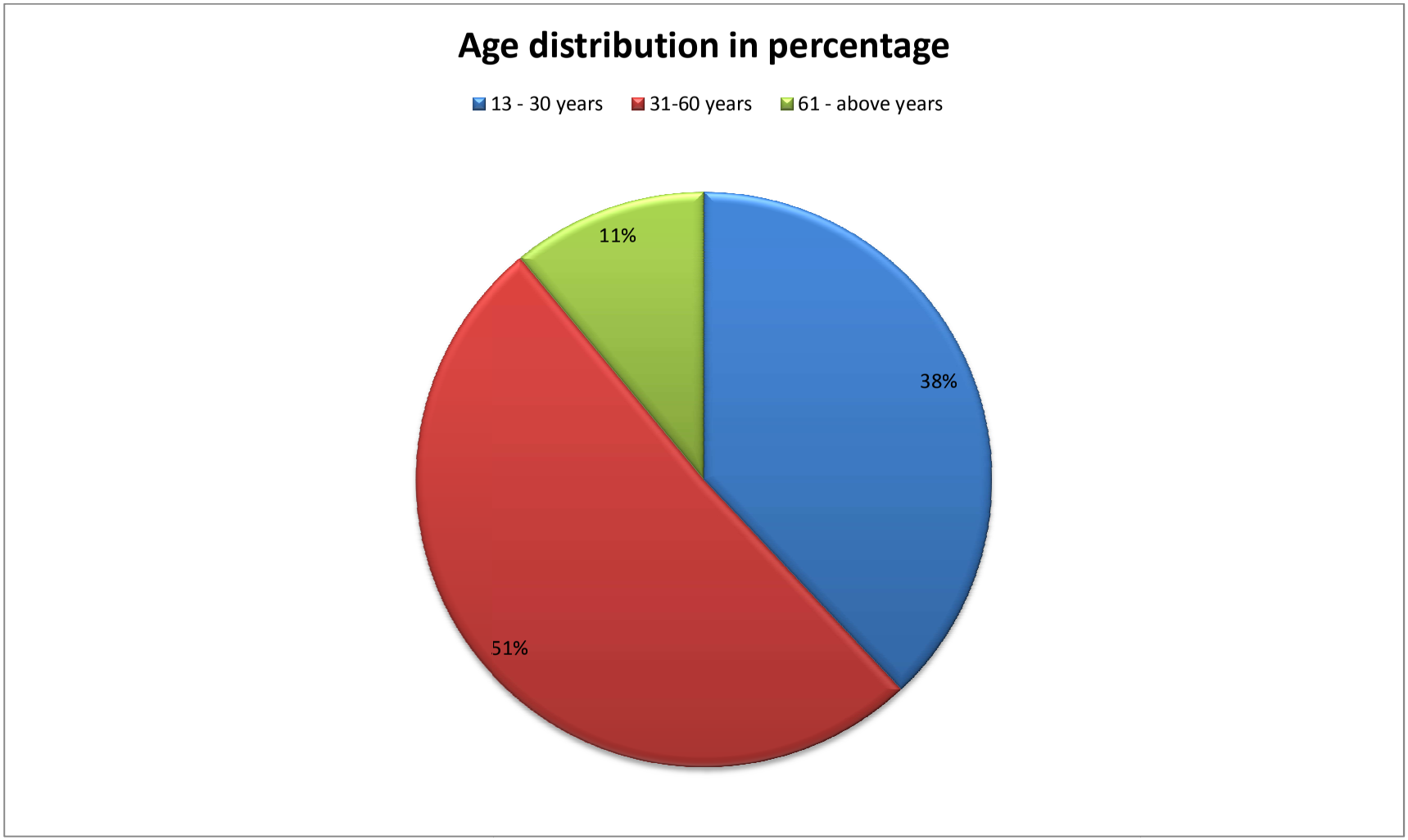


Figure 3: Sex Distribution

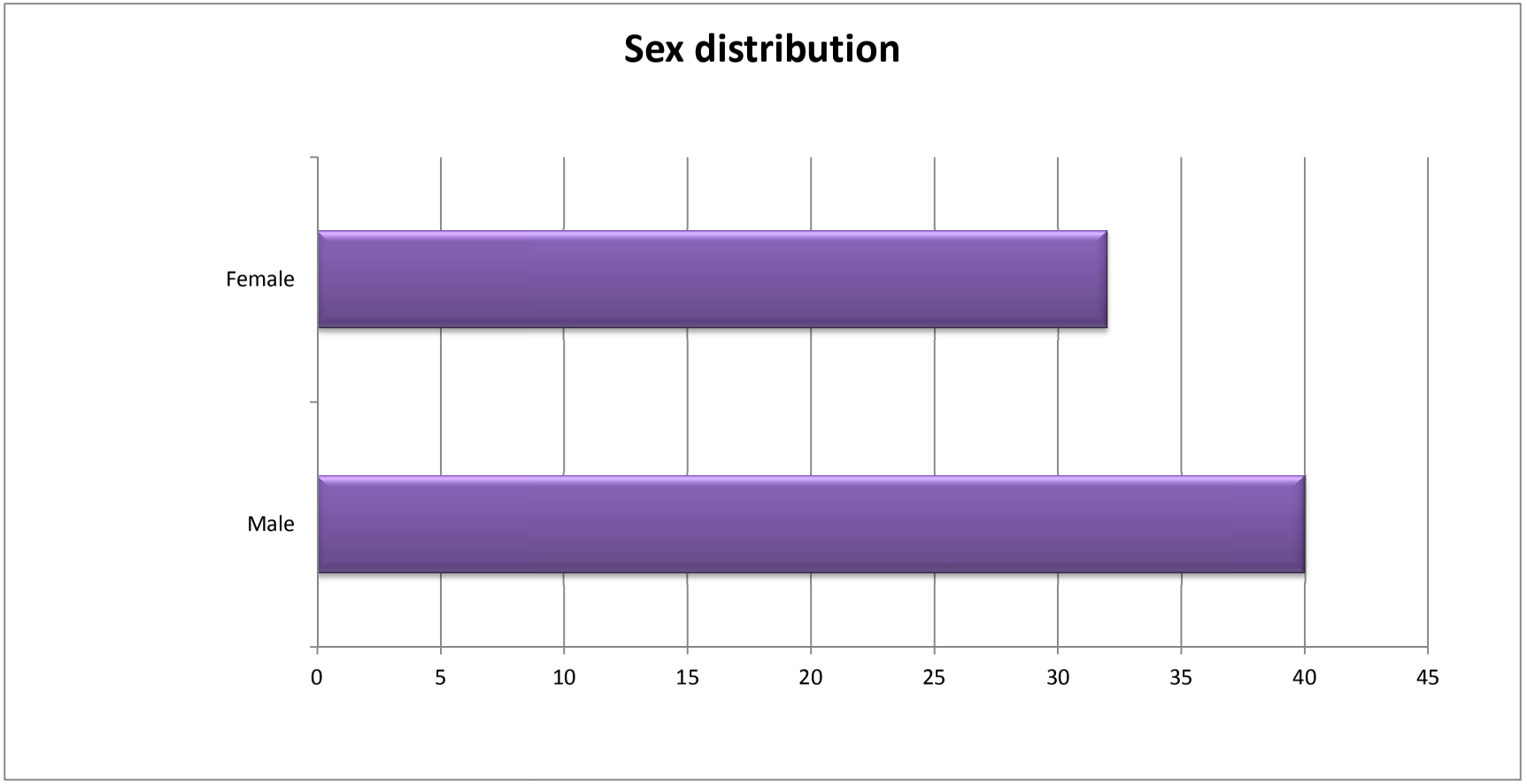


Figure **4 Co-morbidity and Risk Factor Distribution**

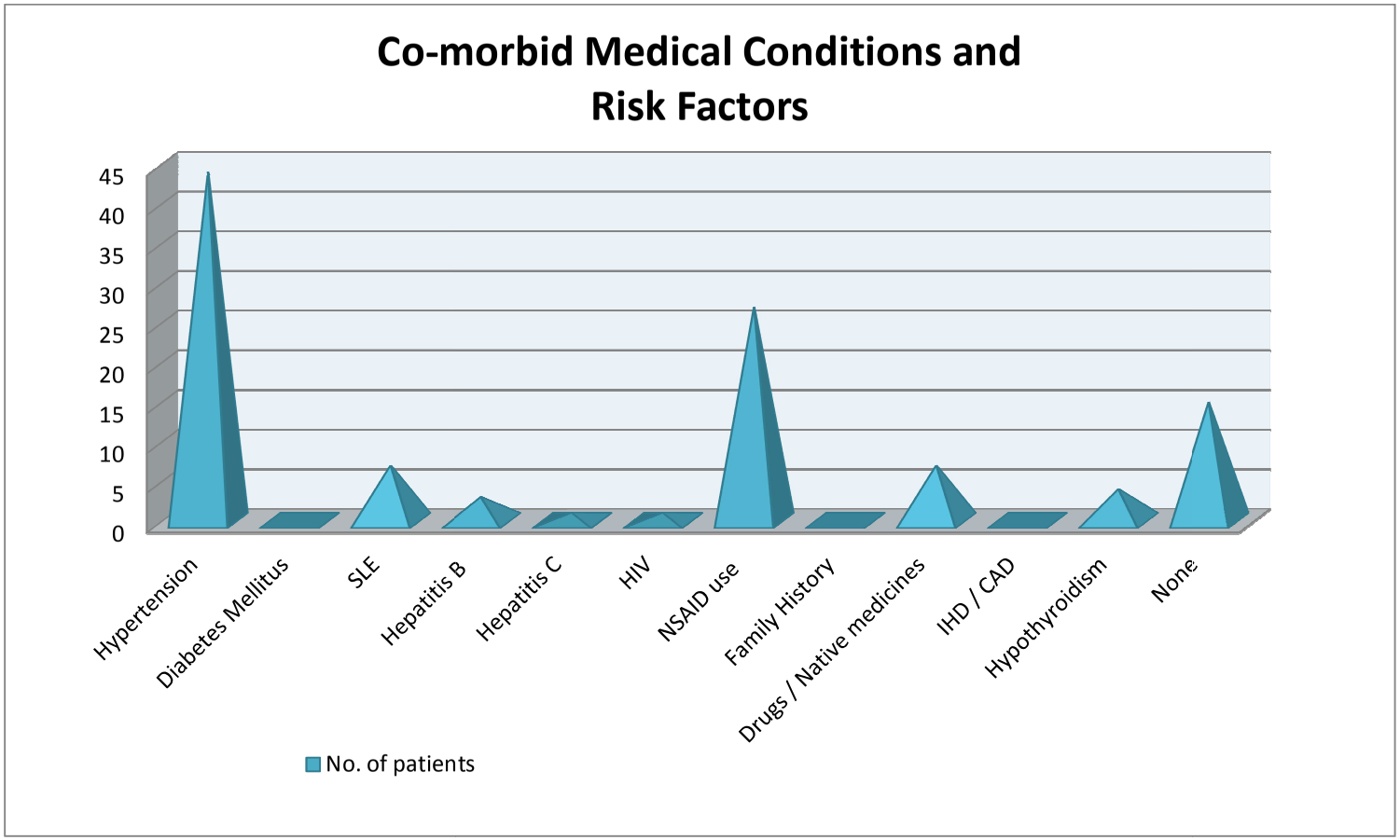


Figure 5. **Risk Factor Distribution(in Percentage)**

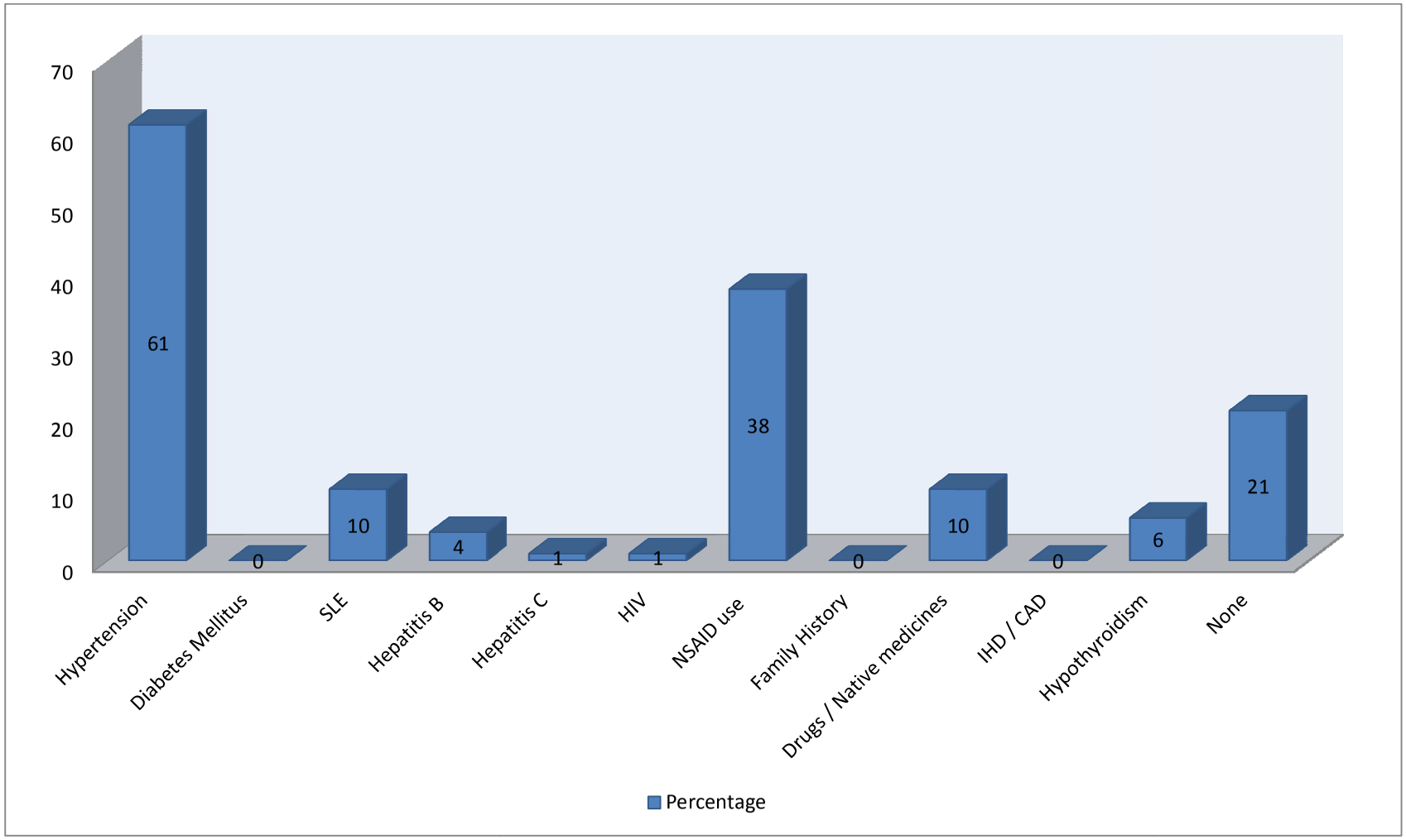


Figure 6. Etiology based on Biopsy Results

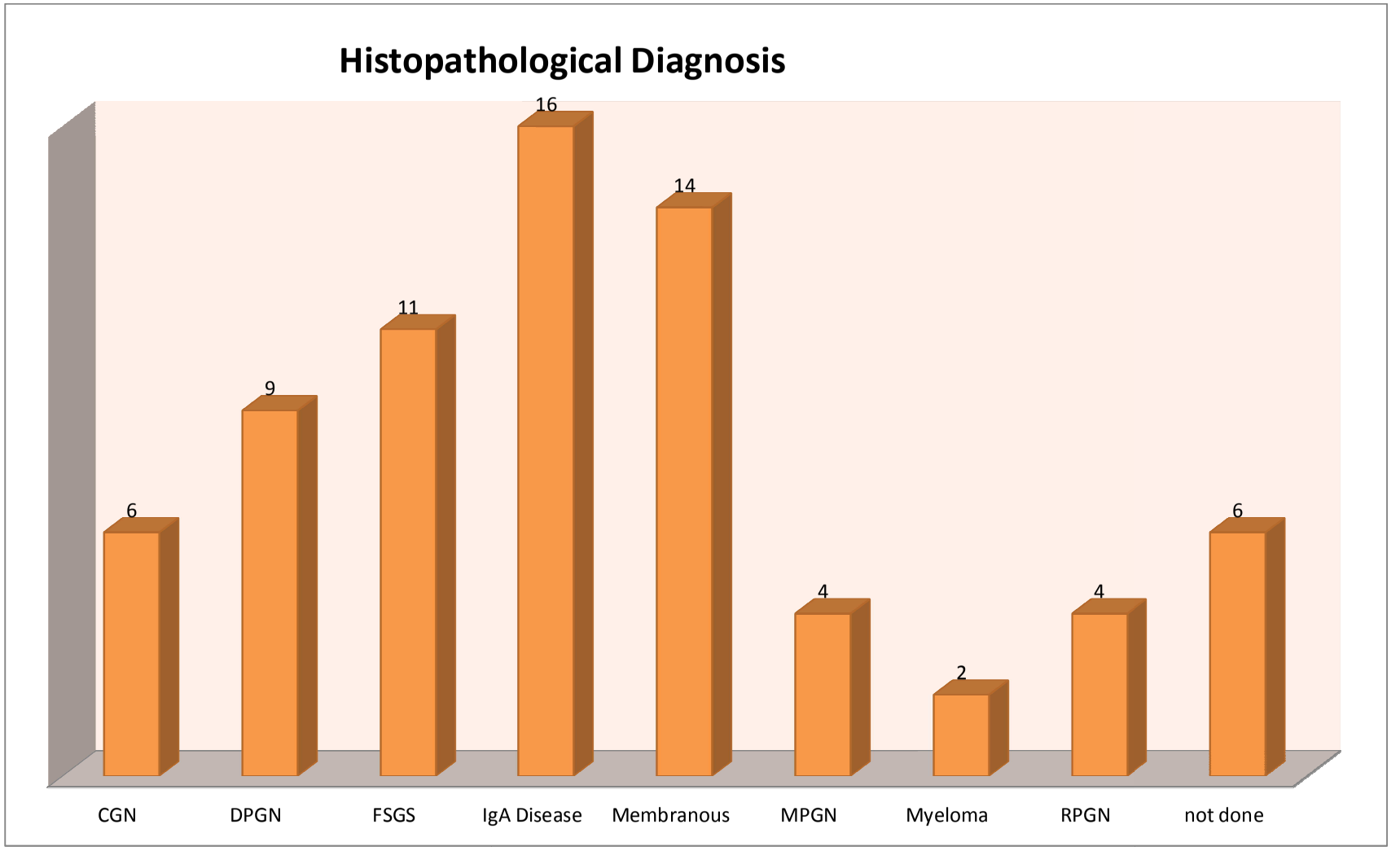


Figure 7: Histopathological Diagnosis

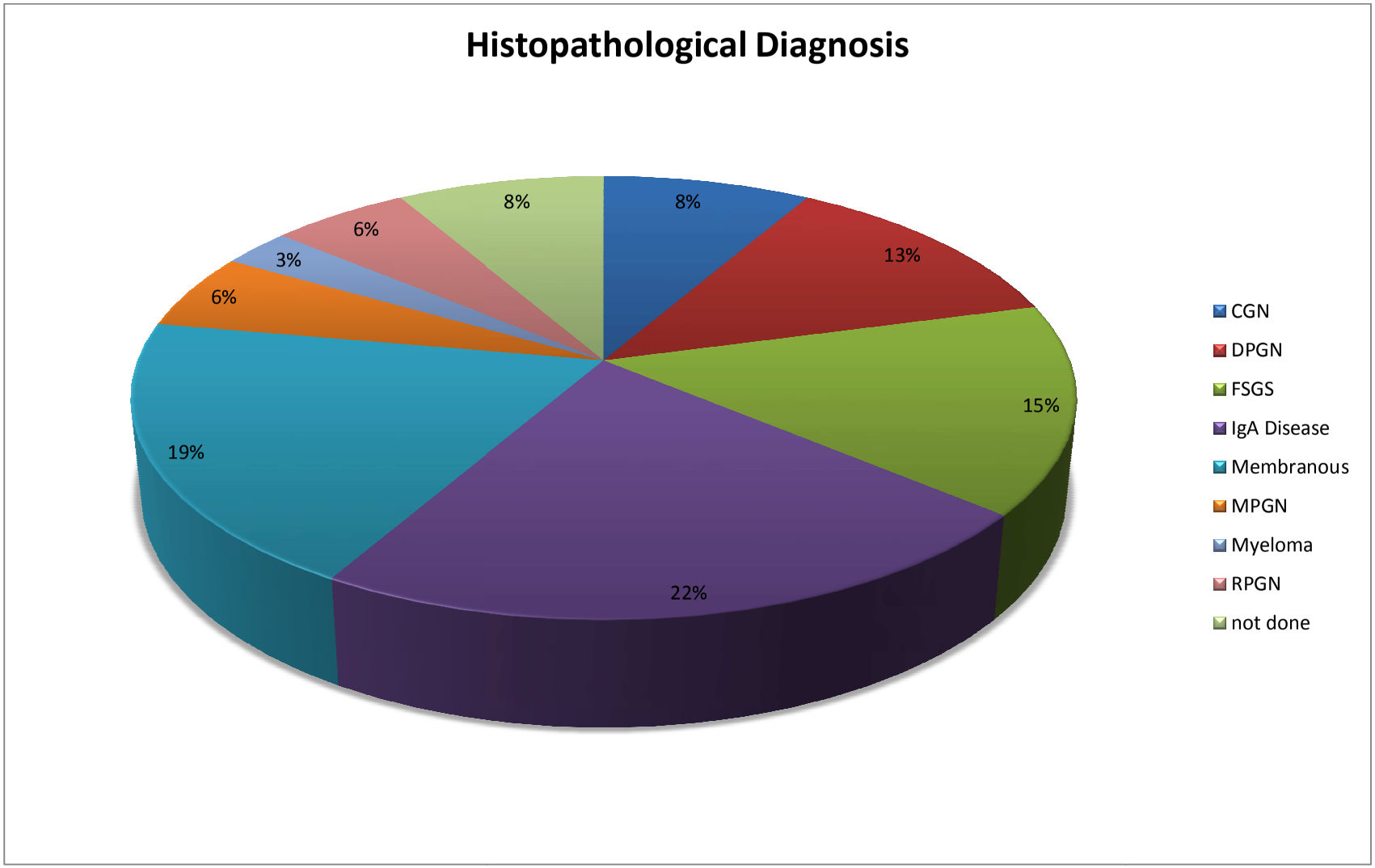


Figure 8. **CKD stage Distribution.**

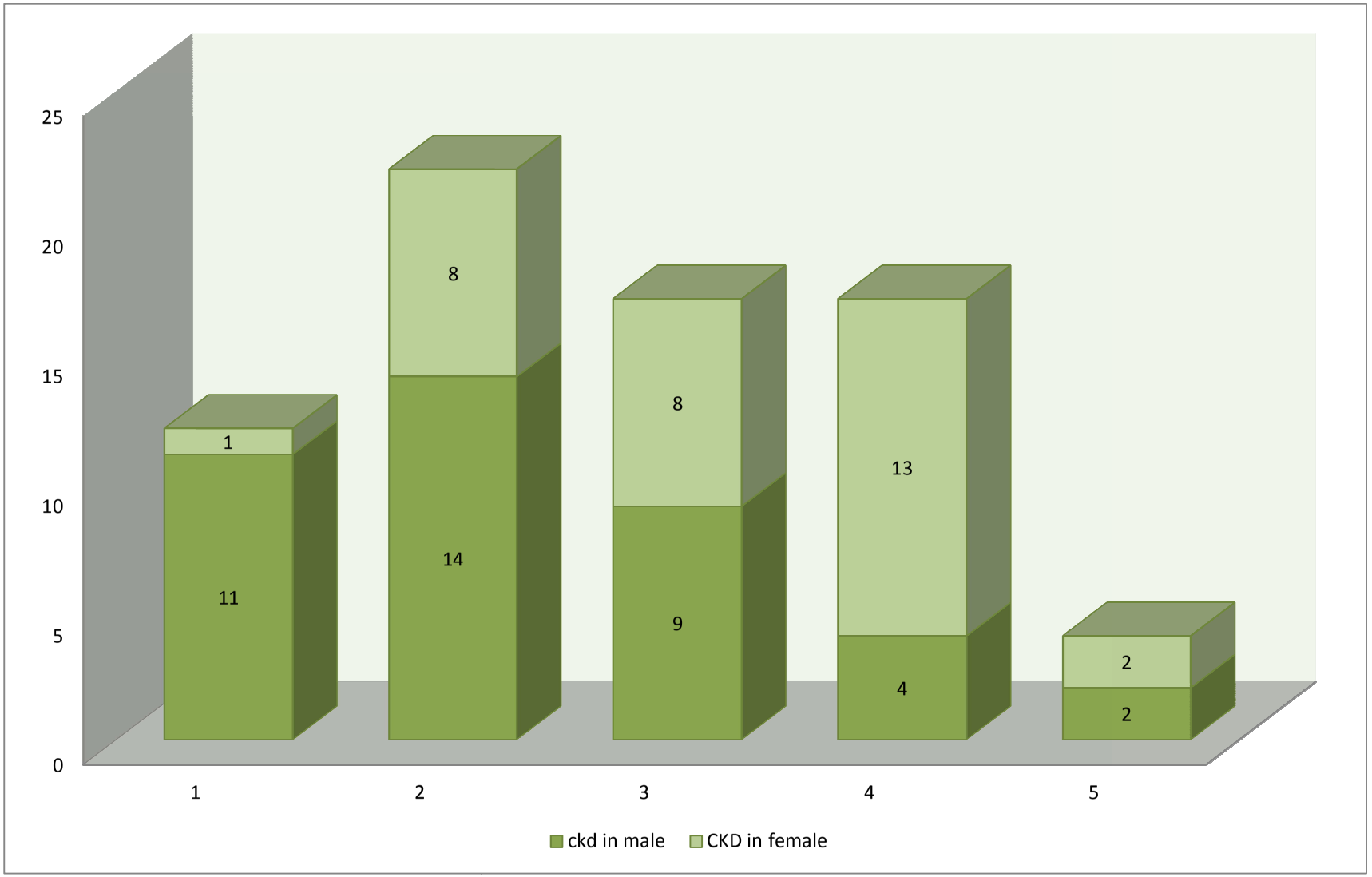


Figure 9. **Distribution of Patients based on CKD Stage(percentage)**

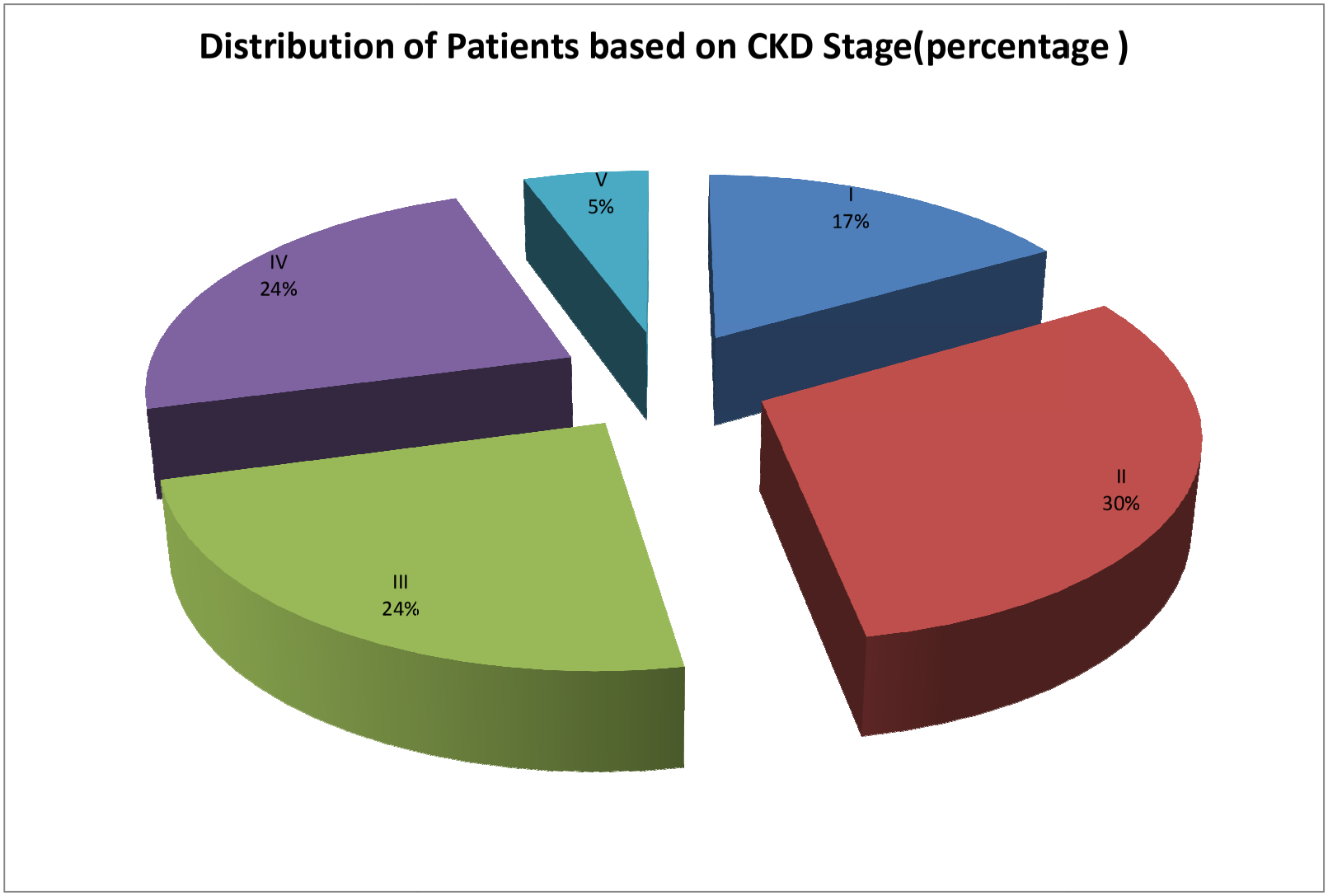


Figure 10: **Scatter plot urine PCR vs. 24 hours urine protein**

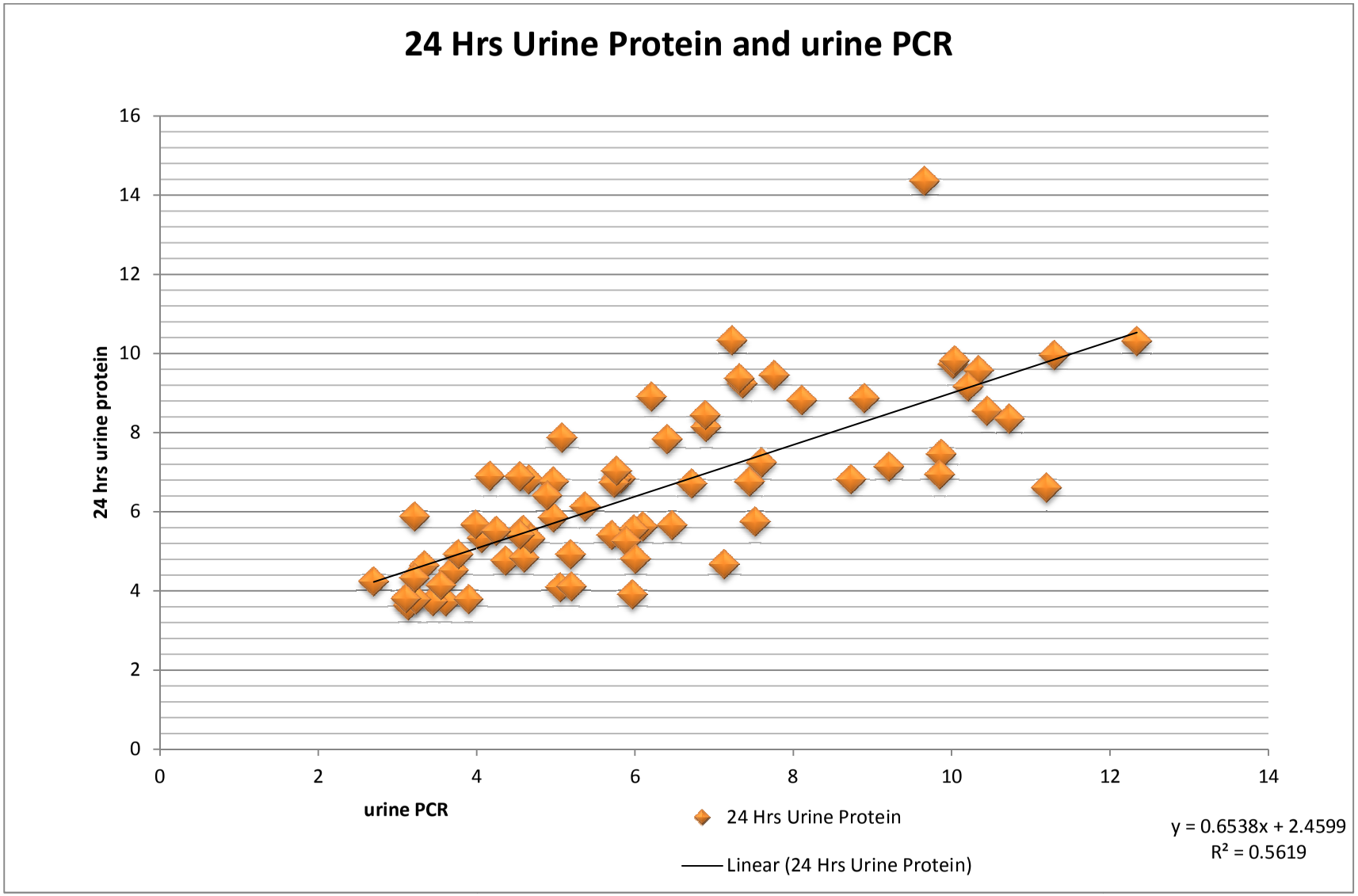


Figure 11: Scatter plot urine ACR vs. 24 hours urine protein

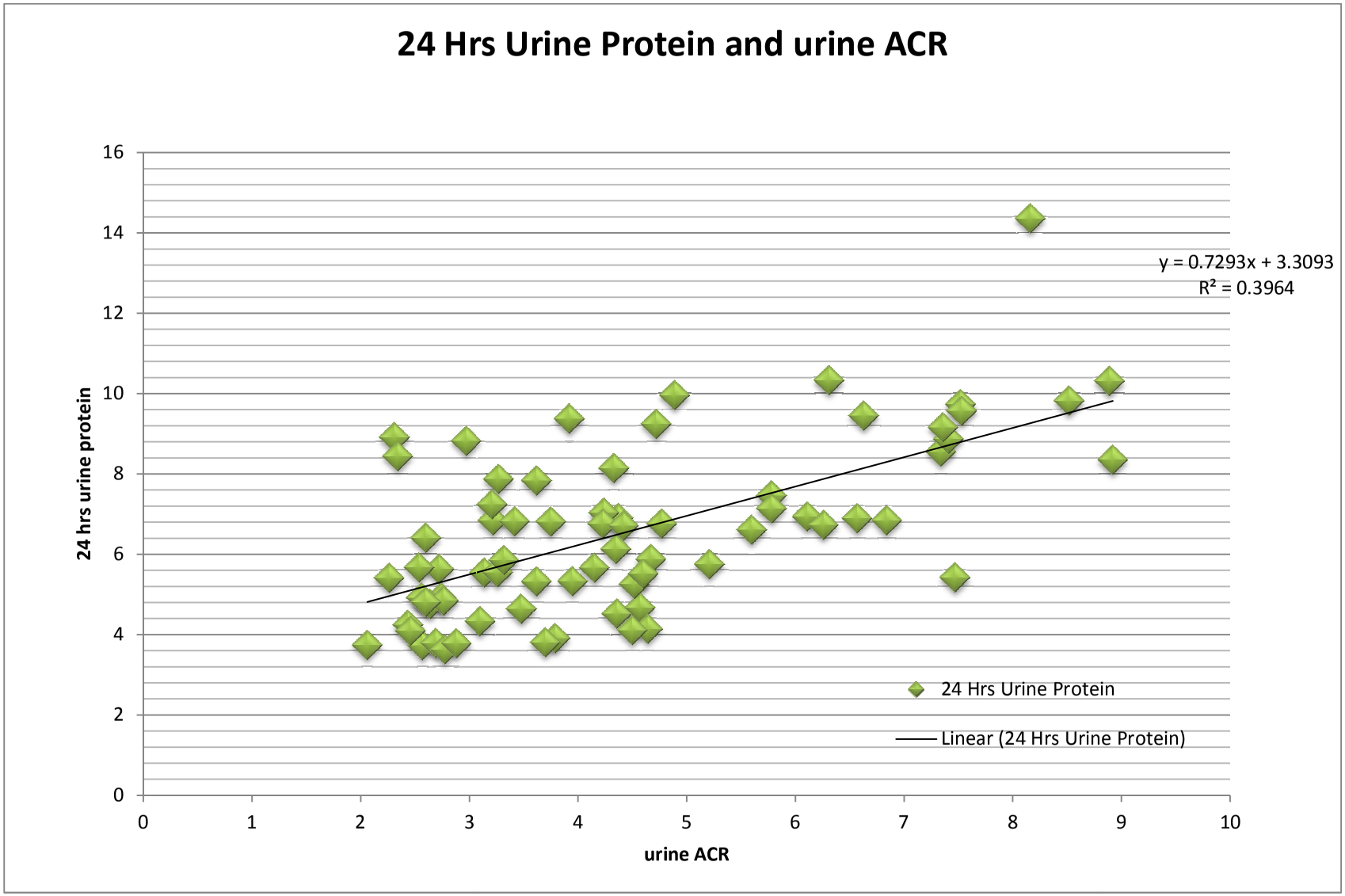


Figure 12. Scatter plot log (urine PCR) vs.log (24 hours urine protein)

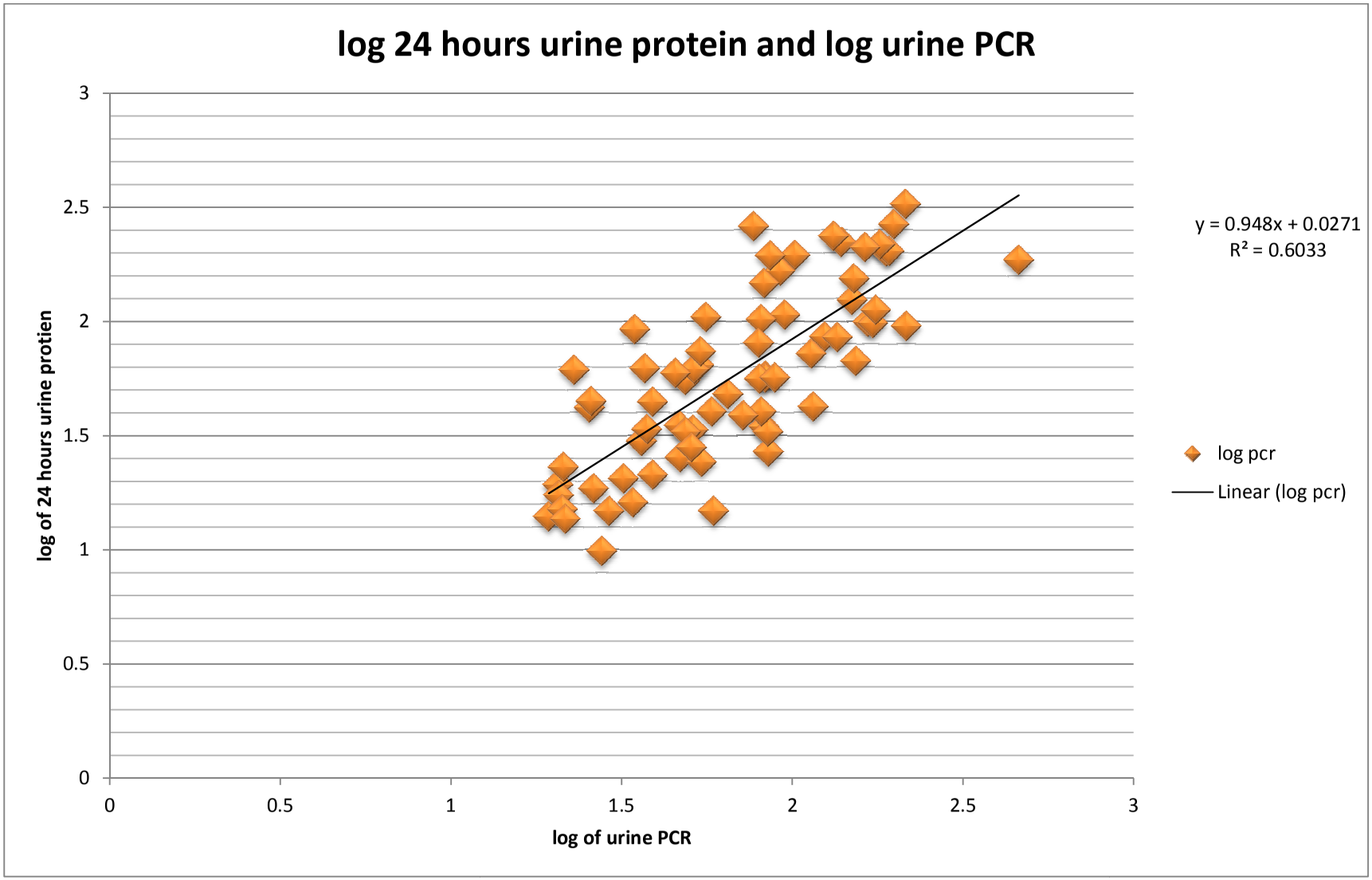
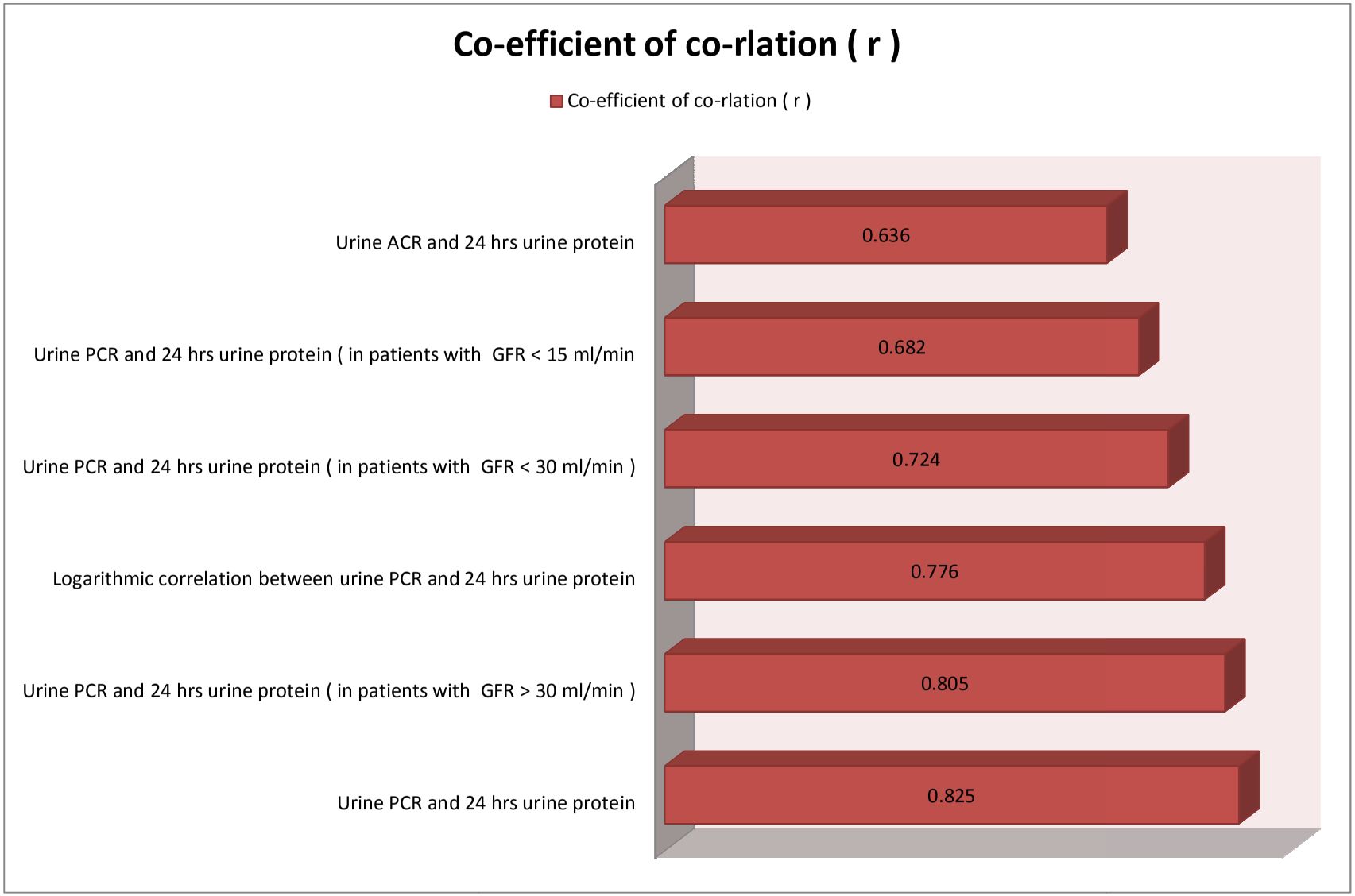


Figure 13. Co-efficient of correlation comparison



DISCUSSION

DISCUSSION :

24 hours urine protein excretion estimation is gold standard for quatitative assessment of proteinuria. This approach, however, is considered by many to be unfeasible in some circumstances, particularly in the outpatient setting, because of the difficulty associated with obtaining a complete collection. Mitchell et al. [22] in their study on the elderly age patients concluded by saying that they had to discard more than 20% of the samples because they were considered to be incomplete. Similarly Chitalia et al.[1] in their study had to dispose off upto 10% of the samples collected for akin reasons. In this study for a total of 72 patients, a total of 83 sample had to be collected as 11 samples (13.3%) had to be discarded.

It is well known fact that there is tremendous intra-day variation in the concentration of urinary protein. The estimates fluctuate by upto 500%. This is the biggest disadvantage of use of a random urine samples or short collection period protein concentration measurements.[23] This variation is thought to be attributable to several factors, including (a) diet, (b) exercise, (c) rate of diuresis, (d) recumbency, and (e) variation in water intake and excretion . The variation may be further exacerbated by pathologic changes in blood pressure and renal architecture.[23] These reasons hence , call for a 24 hours urine collection.

Urine PCR has been suggested as an alternative to 24 hr urine collection. To calculate this ratio first morning urine sample is collected and sent for analysis. The protein and creatinine concentration are detected and then the ratio is obtained. It is expressed in terms of mg of protein per mg of creatinine. This calculation of protein-creatinine ratio on spot urine samples corrects for variations in urinary concentration due to hydration. Newman et al.[59] found that the mean intraindividual variation in the Urine PCR ratio was 38.6%, whereas that of the protein excretion was 96.5%. Koopman et al. [24] had made a similar observation. Based on these observations it is recommended by American Guidelines (K/DOQI clinical practice guidelines for chronic kidney diseases: evaluation, classification and stratification 2002) that the protein-creatinine ratio should preferably be calculated on the first morning urine specimen[2].

According to Deeks et al [25], although several statistical techniques are available, the way that the data are presented are not always readily interpretable by the practicing clinician. However, the most important factor is to have a clear definition of the way in which the test is to be used. He insists on the use of Urine PCR as a screening tool to rule out significant proteinuria. This view has also been shared by Price et al.(2005) [23].

One of the major aspect highlighted by Price et al was the fact that the study population used for conducting such comparative studies that a high prevalence of

proteinuria. Hence the results obtained from such a population could not be extended to the general population as the prevalence of proteinuria in the general population is quite low. Hence the positive predictive value of such a test falls rapidly in such situations.

Craig et al.[26], and Boulware et al. [27],Leeman et. al [63] in a cost-effectiveness analysis, suggested that screening for proteinuria would be useful only in high-risk populations, e.g., older people and persons with hypertension and that Urine PCR might be a reliable indicator of significant proteinuria.

Urine PCR can very well be an alternative to the 24-h collection (Ginsberg et al. 1983).In the experience of Ginsberg et al. (1983)[28] the measurements obtained with this method correlated well with those obtained in the classical way. In fact, all patients with proteinuria of greater than 3.5 g/24 h had a ratio of greater than 3.5 in single voided samples, and all patients with a proteinuria of less than 0.2 g/24 h had a ratio of less than 0.2. it can thus be concluded that the urine PCR is numerically equal to the 24 hours urine protein estimation.

However in this study the urine PCR ranged from 2.7 to 12.44. All the patients except for 1, (98.6%) had a Urine PCR more than 3.1 and 64 (89%) patients had a urine PCR more than 3.5

According to Schwab et al. (1987)[29], patients (representing a broad spectrum of renal diseases, a wide range of proteinuria, and various degrees of reduction in glomerular filtration rate) had Protein-to-creatinine ratios in single-voided urine samples in well correlation with measurements of 24-hour urinary protein. This simple single-voided test is reliable and useful in the screening, assessment, and follow-up of proteinuria and avoids the problems associated with 24-hour urine collection.

Ruggenenti et al. 1998[60] in their study on non-diabetic patients have come a conclusion that urine PCR and 24 hours urine protein estimation correlate very well almost the entire range of glomerular filteration rate and stages of chronic kidney disease.

Zelmanowitz et al[31] reported that proteinuria measurement in a random urine sample was a reliable and a simple method for screening and diagnosing overt diabetic nephropathy.

Price et al.[23] in their systemic meta-analysis from 16 studies investigating urine PCR for quantitative proteinuria assessment in several settings (preeclampsia, renal disease) concluded that the following ranges: sensitivity (69-96%) and specificities (41 - 97%), positive predictive value (46 - 95%) and negative predictive values (45 - 98%), positive likelihood ratios (1.8 and 16.5) and negative likelihood ratios (0.06 and 0.35).

In this study the patients under stage 4 and 5 CKD , had correlation co-efficient (r) values 0.72. However in patients with stage 5 CKD had correlation co-efficient (r) values 0.682. This is less as compared to the correlation coefficient obtained in patients with eGFR > 30 ml/min(0.805) and entire study group as a whole (0.825).

There are few studies that have highlightened this aspect of use of urine PCR in estimation of 24 hours urine protein estimation.[32,33,34,35]

Siwach et al [32] found that in patient with normal or mild to moderately impaired renal function the product of PCR and estimated daily urinary creatinine excretion positively correlated well with the estimated 24 hours urine protein (r = 0.88 and 0.99), but poorly correlated in patients with advanced renal failure (r = 0.56)

Mohan et al [34] studied the correlation between the expected 24 hours urine protein calculated from spot urine protein – Creatinine ratio and the estimated 24 hours urine protein in type 2 Diabetes. The positive correction was good, but was less with increasing degree of proteinuria.

Sharma et al [35] studied the correlation between the Protein-Creatinine ratio in spot urine sample with 24hours urine protein with varying degree of renal dysfunction and concluded a good positive correlation in patients with advanced

renal failure. Correlation co efficient (r) values were 0.889, 0.788, 0.595 in patients with serum Creatinine < 1.5 mg/dl, 1.5-4 mg/dl, > 4 mg/dl respectively

Goldman et. al [33], Morales et. al(2004)[68] found that the possible reason for poor correlation is these patients is that with progression of renal failure the urinary Creatinine excretion falls especially after serum Creatinine exceeds 6 mg/dl.

Many studies have concluded that urine PCR is numerically equal to the 24 hours urine protein excretion. However the studies also conclude that this relation gets weaker as the GFR worsens and heavy proteinuria. Ruggenenti et al. (1998)[60] suggested that the logarithmic values of 24 hours urine protein and Urine PCR co-relate equally well and maintain the relationship even in heavy proteinuric patients.

In this study, a scatter plot of log 24 hours urine protein and log urine PCR showed that there is excellent correlation between the two variables and the values are numerically almost equal. This is a further proof to say that urine PCR can be reliably used as an estimate of 24 hours urine protein even in patients with nephrotic range proteinuria.

Table 13. **Comparison between various studies**

Name of the Study	Comparison between 24 hours urine protein and …	No. of Patients	r value	p value
Quadri et al., 1994 [43]	Urine PCR	75	0.92	<0.0001
Young et al., 1996 [44]	Urine PCR	45	0.8	<0.001
Robert et al., 1997 [45]	Urine PCR	71	0.94	<0.001
Saudan et al., 1997 [46]	Urine PCR	100	0.93	<0.001
Ramos et al., 1999 [47]	Urine PCR	47	0.94	Not stated
Evans et al., 2000 [48]	Urine PCR	51	0.95	<0.0001
Rodriguez-Thompson et al., 2001 [49]	Urine PCR	138	0.8	<0.001
Durnwald and Mercer, 2003 [50]	Urine PCR	220	0.64	<0.0001
Al et al., 2004 [51]	Urine PCR	185	0.56	<0.01
Yamasmit et al., 2004 [52]	Urine PCR	42	0.95	<0.001
Combs et al., 1991 [53]	Urine PCR	329	0.98	<0.0001
Ginsberg et al., 1983 [28]	Urine PCR	46	0.97	Not stated
Schwab et al., 1987 [29]	Urine PCR	101	0.96	Not stated
Abitbol et al., 1990 [54]	Urine PCR	64	0.95	<0.001
Dyson et al., 1992 [55]	Urine PCR	148	0.77	<0.001
Steinhauslin et al., 1995 [56]	Urine PCR	318	0.93	<0.001
Chitalia et al., 2001 [1]	Urine PCR	170	0.97	Not stated
Torng et al., 2001 [57]	Urine PCR	289	0.79	<0.0001
Ralston et.al.,1988 [58]	Urine PCR	102	0.92	<0.001
Mitchell.et.al.,1993[10]	Urine PCR	52	0.98	<0.0001
Morales et. al 2004 [68]	Urine PCR	43	0.91	<0.001
Xin et. al 2004[69]	Urine PCR	72	0.823	<0.001
Rodby et. al [67]	Urine PCR	33	0.90	Not stated
Boler et. al[73]	Urine PCR	54	0.9936	<0.001

In this table it is very clear that there is a definite excellent correlation between 24 hours urine protein and urine PCR and this is true over a wide range of proteinuria and varying renal function. The studies included in the above table have a majority population of pregnant women wherin urine PCR was done to rule out significant proteinuria which is associated with pre-eclamsia. However it also includes 2 studies done on post renal transplant patient and patients attendin nephrology out patient departments each.In our study there were similar results.

Urine Albumin- creatinine ratio

Similar to calculation of urine PCR , urine ACR can also be calculated for spot first morning urine samples. The urine albumin-creatinine ratio on random urine samples can be used (Assadi et. al 2002)[36]in diabetic patients to assess the grade of nephropathy. With this method microalbuminuria is defined as a ratio of 30-300 μg albumin/mg creatinine.[74]

Urinary albumin concentration correlates well with urinary total protein and also with 24 hour urinary protein over a very wide range of the level of proteinuria. It was hence being investigated as a marker of 24 hours urine protein.[75]

Newman et al[59] concluded that albumin concentrations increased significantly after vigorous activity and there is considerable diurnal variation .

The increase was almost eliminated when the albumin result was divided by the creatinine concentration suggesting that a decreased urine flow and not increased glomerular permeability causes an increase of post-exercise albuminuria.First morning sample was collected to further reduce this diurnal variation.

Though this test is especially useful in diabetic patients , it can also be used to assess pathological proteinuria in non diabetic patients. According to Price 2005,[23] considerable disparity in the measurement of total protein in urine is most probably is a result of differences in the analytical specificities of the methods used as well as changes in the calibration methods. This may have contributed to the variation in the diagnostic performance among the studies. It has been suggested that the measurement of albumin might offer a means of reducing methodologic variation. It also has the potential for increasing the clinical diagnostic sensitivity.

The most common methods to measure micro albuminuria are radioimmunoassay (Woo et al. 1978)[62], enzyme immunoassay (Fielding et al. 1987)[37], nephelometric (Stamp 1988)[38], and immune-turbidimetric (Shukla et al. 1988)[39]. In this study quantitative estimation of albumin was done using immune-turbidimetric method. These detect can albumin at concentration of 10-20 mg/l, and have a 70-90 per cent specificity.[40,41,42]

Only few studies exist for use of urine ACR as an estimate for 24 hours urine protein excretion.Guy M et. al [61] conclude urine ACR accurately predicted an abnormal 24 h urine albumin. He also concluded that urine ACR can be used in predicting proteinuria by careful choice of cut-offs in patients with kidney disease to rule in or rule out abnormal 24 h losses of protein and albumin. Early morning urine sample as well as random samples can be used as surrogates for 24 hours urine protein excretion. However , this study primarily concentrated its efforts on use to urine ACR to rule out significant albuminuria.

In this study there was no significant difference between 24 hours urine protein excretion and urine ACR. There also was an excellent correlation between 24 hours urine protein excretion and urine ACR($r = 0.636$). However the correlation is weaker as compared to that with urine PCR($r = 0.825$). the strength of relationship as calculated using a scatter plot was good but not as good enough as that seen with urine PCR and 24 hours urine protein. As the correlation is considerable weaker the correlation between 24 hours urine protein excretion and urine ACR in patients with reducing renal function would be expectedly lower. Accordingly the values suggested the same. The co-efficient of correlation between urine ACR and 24 hours urine protein was 0.672 and 0.503 in patients with GFR > 30ml/min and GFR < 30ml/min respectively. Hence it can be concluded that urine

PCR is a better index for estimation of 24 hours urine protein excretion as compared to urine ACR.

Further prospective studies will be required in specific patient populations to validate these conclusions.Wilson et. al [76] and Kim et. al[77] have also highlighted the use of protein-osmolality ratio as a reliable marker to esaimate 2 hours urine proein excretion. However there are very few studies to confirm this.

Comparison with the Indian Scenario of Nephrotic Syndrome:

S Siddappa and associates[79] in a recently published data on 400 renal biopsy at a centre in Hyderabad, South India show the recent increasing trend of increasedincidence of IgA disease and conclude that IgA nephropathy is the most common cause of primary glomerular disease. the most common presentation of IgA was a nephrotic range proteinuria followed by chronic renal failure. Almost all patients with IgA disease had atleast one episode of documented microscopic (>5 RBC/ Hpf) or macroscopic hematuria. The mean proteinuria of the IgA group ranged from 3.54 – 5.56 in the common subtypes of IgA nephropathy.The mean age was 36.6 years and male preponderance was seen. They also concluded that there is undue genetic susceptibility of the Asian population to both the development of IgA disease and its complications.

U. Das and colloegues [80] in another recently concluded Indian study, a collective experience of renal biopsy results over 19 years at a single centre was analysed. They conclude that nephrotic syndrome remains the most common indication for a renal biopsy(49%). The most frequent cause of Nephrotic syndrome in the patients above 20years of age was Minimal Change Disease followed by Membranous nephropathy and then FSGS. Authors said that there was change in policy for renal biopsy and immunoflorescence staining over the years and probably a selection bias which may have confounded the results partially. They also concluded that for Membranous Nephropathy the mean age was 40, sex ratio 2.3:1 and a mean proteinuria of 4.8 gm/day. Similarly for IgA disease the mean age was 26 , sex ratio 3.3:1 and a mean proteinuria was 2gm/day. In FSGS group the mean age was 25, sex ratio 2.25:1 and a mean proteinuria was 3.3 gm/day. In this study they have also highlighted to the fact that among all the IgA nephropathy patients 44.6% presented with nephrotic range proteinuria.

According to Reshi AR et al [81], who recently published their data over nephrotic syndrome in single centre in Kashmir,North India , the most common cause of nephrotic syndrome in adults was minimal change disease followed by FSGS , MN and IgA nephropathy. This study points to the regional variation in the etiology in India itself.

The largest data from a single centre in India came from Vellore,South India where the last 30 years(1986-2002) with analysis of 5415 native kidney biopsy results[82]. Nephrotic syndrome was the most common indication (65%) and among the entire group the most common cause was Mesangio-proliferative glomerulonephritis, The prevalence of FSGS was 17%. IgA nephropathy prevalence was 8.6%.Minimal Change disease and membranous nephropathy prevalence was 11.6 and 9.7 .% respectively.

In our study however the most common of adult onset non diabetic nephrotic range proteinuric patients was IgA nephropathy(22%) followed by membranous nephropathy(19%) and then FSGS(15%).

Drawbacks of this study:

Some authors have stressed upon the fact that the mere performance of a regression analysis and then calculating a high correlation coefficient (r) may not always enable a physician to make a reliable decision of substituting 24 hours urine collection with a urine PCR [1]. Thus, the high degree of association assessed by high coefficient of correlation values (almost approaching 1)between the Urine PCR ratio and the 24-h protein excretion does not necessarily give dependable information on whether use of the ratio in a random sample will enable clinicians

to reduce their dependence on the 24-h urine collection. There is no follow up and sequential PCR and 24 Hours urine protein comparison .The sensitivity urine PCR to accurately detect proteinuria in nephrotic range was not calculated. There is a possibility of variation in results if a different method was used for the quantitative estimation of proteinuria. Cost benefit analysis was not done.

CONCLUSION

CONCLUSIONS :

1.PCR in the first morning urine sample is found to be an useful index for quantification of proteinuria in patients with heavy proteinuria and varying degrees of renal dysfunction.

2.There was good positive correlation between spot urine PCR and 24 hours estimated protein.

3.The correlation was maximum in patients with GFR > 30ml/min.

4.The positive correlation was least in patients severe renal dysfunction (Stage 5 CKD).

5.There was no significant difference between expected and estimated 24 hours urine protein.

6.Urine PCR is easy to perform, inexpensive and less time consuming method for measuring of proteinuria. It can thus be used in the out patient setting for screening and quantification of proteinuria.

7. Urine ACR in the first morning urine sample is found to be an useful index for quantification of proteinuria in patients with heavy proteinuria and varying degrees of renal dysfunction.

8. There was good positive correlation between spot urine Albumin-Creatinine ratio and 24 hours estimated protein.

9. Urine PCR correlates better than urine ACR in quantitative estimation of 24 hours urine protein estimation

10. Log Urine PCR is almost equivalent to Log of 24 hours urinary protein.

SUMMARY

SUMMARY:

24 hours urine protein estimation has been standard to quantify proteinuria. However it is cumbersome, may have to collection errors, required good patient compliance, and result in delay in diagnosis. A meta-analysis showed that urine PCR is an useful index to estimate 24 hours urine protein excretion .This study was undertaken to find if the urine PCR from first morning urine sample could reflect the amount of protein in 24 hours in patients with nephrotic range proteinuria. 72 patients with proven nephrotic range proteinuria and varying degree Creatinine clearance were investigated. An excellent correlation was found between 24 hours urine protein and protein- Creatinine ratio. However correlation weaken as the GFR worsens and is weakest in patients with stage 5 CKD. The correlation between urine ACR and 24 hours urine protein is considerably weaker than that with urine PCR. This study supports the use of a single voided Protein-Creatinine ratio to predict 24 hours urine protein. Use of urine PCR avoids collection errors, less time consuming and is suitable for out patient departments.

ABSTRACT:

Background and Objective :

Quantitation of proteinuria by 24-hour urine collection is a conerstone of monitoring disease activity in patients with patients with nephrotic range proteinuria(>3.5 gms/day). Such collections, however, are often inaccurate, inadequate, tedious, time consuming. Up to 20 % of 24 hours urine collection samples get discarded because of inadequate or incorrect collection.[1] The urine PCR (protein creatinine ratio) and urine ACR (albumin creatinine ratio) corrects for variations in urinary protein concentration due to hydration and is not affected by a decrease in urine output in patients with renal insufficiency. It is far more convenient than timed urine collections. There are several studies showing the correlation in sub-nephrotic range proteinuric patients. But there are comparatively fewer studies correlating these variables in the only nephrotic range. Also very few studies have compared the efficacy of urine PCR with urine ACR to quantify 24 hours proteinuria.

Method :

72 patients with proven nephrotic range proteinuria with varying degree of renal dysfunction were included in this study. First morning spot urine sample was collected for the estimation of urine PCR and urine ACR. Then the values were compared.

Results :

There is significant correlation not only between 24 hours urine protein and protein-Creatinine ratio (r = 0.825) (P < 0.001) but also between 24 hours urine protein and albumin -Creatinine ratio (r = 0.636) (P < 0.001). The correlation between urine PCR and 24 hours urine protein becomes weaker as the Glomerular Filtration Rate (GFR) worsens and is weakest at stage 5 chronic kidney disease(CKD) (r = 0.682).The logarithm value of 24 hours urine protein is numerically equal to logarithm value of urine PCR(r=0.876)

Conclusion:

Spot morning urine samples for urine PCR and urine ACR(though less precise) are precise indicators of proteinuria and represents a simple, rapid procedure in establishing severity of heavy proteinuria.

Key Words :

Proteinuria , urine protein - creatinine ratio, urine albumin - creatinine ratio, quantitative estimation

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PROFORMA

Name :

Age / Sex : **Marital Status :**

Educational Status : **Occupation :**

Address :

Clinical features:-

Facial puffiness
Generalised Edema
Urine frequency
Dysuria
Burning Micturation
Altered sensorium
Loss of consciousness
Pleural Effusion
Ascitis
Fever

Past History:-

SLE……(duration…….;treatment……)
Diabetes Mellitus…….(duration……treatment……..)
Hypertension…….(duration …….;treatment…….)
IHD…….....(duration……..;treatment……)
Stroke……(duration……..;treatment……)
Renal disease
Other Autoimmune Disease
Liver disease
Thyroid dysfunction

Other Significant Past History :

Personal History:

Diet - vegetarian / non-vegetarian /mixed / fruits / fast food
Apetite -good /poor
Bowel - normal / constipation / loose stools
Bladder - normal / polyuria / oliguria
Sleep - normal / reduced
Mental Stress- low / mod / high
Smoking - smoker
Ex- smoker - quit since …….

Never a smoker
Alcohol: - duration
-unit
Tobacco -
Exercise -

Treatment History :

Family History: (Y / N)

Coronary Artery Disease
Diabetes
Renal Disease
Hypertension

General Physical Examination:

Anthropometric measurements :

Ht (in cm) -
Wt (in kgs) -
BMI (kg /m2)
TBSA

Pallor
Icterus
Cyanosis
Clubbing
Lymphadenopathy
Pedal oedema

Vitals:

Temperature (in F)
Pulse (/min)
BP (mm of Hg)
Pulse pressure (mm of Hg)
Respiratory Rate

Systemic Examination :

CVS :

RS:

PA:

CNS:

ECG

X ray chest :

Hb	
TC	
DC	
ESR	
Platelet	

Urine	
Protein	
Sugar	
Cells	
Casts	

Serology	
HIV	
HBsAg	
Anti- HCV	

Urea	
Creatinine	
S. Sodium	
S. Potassium	
S. Calcium:	
S. Phosphorus:	

Urine Analysis:	
24 hours urine protein estimation	
No. of attempts	
Urine Creatinine concentration	
Spot urine Protein – Creatinine Ratio	
Spot urine Albumin – Creatinine Ratio	
Spot urine Protein – Osmolality Ratio	

Renal Biopsy :

CONSENT FORM(English)

1). I agree to participate in the study entitled 'CORRELATION BETWEEN THE SPOT URINE PROTEIN CREATININE RATIO, SPOT URINE ALBUMIN CREATININE RATIO AND 24 HOURS URINE PROTEIN ESTIMATION IN PATIENTS WITH NEPHROTIC RANGE PROTEINURIA'

2).I confirm that I have been told about this study in my mother tongue and have had the opportunity to ask questions

3). I understand that my participation is voluntary and I may refuse to participate at any time without giving any reasons and without affecting my benefits.

4). I agree not to restrict the use of any data or results that arise from this study.

Name of the participant :

Sign / Thumb print :

Sign of the Investigator

S. No	Name	Age	Sex	Wt	Ht	risk factors	s.Urea	s. Cr	RBS	eGFR	CKD	24 hr vol.	ACR	ur cr conc	PCR	24Hrs U Pr	Biopsy report	dipstic	attmpt
1	Saribabanu	52	F	82	162	htn,nsaid	29	2.2	112	36.44	4	2100	4.36	0.58	3.71	4.51	FSGS	3	1
2	Jaamal	19	M	42	166	htn	146	8.3	115	8.504	5	650	5.78	1.82	9.87	7.45	IgA Nephropathy	3	1
3	Ramchandran	75	M	57	169	htn,nsaid	66	2	125	25.73	3	1350	8.162	1.1	9.66	14.35	Membranous	3	1
4	Velmurgan	39	M	59	162	drug	23	0.8	97	103.5	1	1550	2.437	1.01	2.7	4.23	FSGS	3	1
5	Vishvalingam	33	M	58	171		33	1.5	113	57.46	2	1100	4.37	1.5	4.17	6.89	IgA Nephropathy	3	1
6	Vijaylakshmi	22	F	42	168	htn,sle	107	4.4	75	12.52	4	3100	4.57	0.43	7.13	4.66	DPGN	3	1
7	Raja	31	M	62	168	htn	121	8.2	98	11.45	5	1150	6.31	1.24	7.23	10.32	Membranous	3	1
8	Mahalakshmi	15	F	39	152		19	0.9	110	60.19	2	1650	2.56	0.6	5.19	4.91	FSGS	3	1
9	Mariamma	54	F	72	162	nsaid	33	1.3	97	52.92	3	1700	6.84	0.69	5.82	6.83	DPGN	3	1
10	Sasikumar	20	M	57	162	htn	36	1.7	78	55.88	2	1150	4.72	1.09	7.36	9.23	Membranous	3	1
11	Krishnan	45	M	53	166	htn,nsaid	48	1.2	99	58.28	2	1750	3.75	0.86	4.66	6.8	IgA Nephropathy	3	2
12	Duniyarani	23	F	43	165	htn,sle	98	4.5	110	12.42	4	1550	3.62	0.79	6.41	7.82	Membranous	3	1
13	Sasikumar	20	M	58	162		16	0.9	92	107.4	1	1750	2.972	0.62	8.11	8.81	Membranous	3	2
14	Kesavan	40	M	59	166	htn	56	2.4	123	34.14	3	2150	3.14	0.56	4.59	5.53	FSGS	3	1
15	Lakshmi	27	F	49	156	htn,hypothroid	28	0.9	78	68.36	2	2100	2.264	0.45	5.71	5.4	FSGS	3	1
16	Sathyameena	32	F	51	160	htn	62	2.6	101	23.54	4	1700	7.34	0.64	10.5	8.54	IgA Nephropathy	3	2
17	Velmurgan	38	M	61	161	drug	28	1.2	104	72.01	2	950	2.31	1.51	6.21	8.9	CGN	3	1
18	Shajahn	39	M	48	166	htn	112	3.4	112	19.8	4	2150	3.27	0.72	5.08	7.86	FSGS	3	1
19	Krishnan	78	M	65	172	htn,nsaid	17	0.9	106	62.19	2	2700	2.74	0.5	3.77	4.91	not done	3	1
20	Raagaiah	35	M	63	177	hiv	18	0.9	80	102.1	1	1250	3.22	1.51	5.77	6.83	FSGS	3	1
21	Vijaykumar	42	M	79	168	htn,nsaid	17	0.9	115	119.5	1	1550	2.64	1.03	4.37	4.75	IgA Nephropathy	3	2
22	Thangadurai	34	M	67	174		22	0.9	119	109.6	1	1550	4.67	1.21	3.22	5.87	CGN	3	1
23	Kausalya	70	F	51	155	htn,hbv,nsaid	29	1.2	85	33.06	3	2300	2.719	0.4	6.1	5.62	MPGN	3	2
24	Farxana	25	F	45	158		17	0.9	117	63.89	2	2150	3.21	0.44	7.6	7.23	MPGN	3	2

25	Senbegum	26	F	47	167	htn,sle	69	4.9	112	12.15	4	2350	3.92	0.52	7.32	9.36	Membranous	3	1
26	Lakshmi	60	F	62	153	htn,hypothyroid,nsaid	73	1.7	93	32.42	4	1750	2.76	0.6	4.6	4.83	DPGN	3	1
27	Srilakshmi	35	F	72	167		87	4.5	87	18.67	4	750	3.95	1.74	4.07	5.32	IgA Nephropathy	3	1
28	Fathimabeevi	74	F	62	162	nsaid,drug	21	1.1	160	41.33	3	2200	3.26	0.42	5.99	5.54	Myeloma	1	1
29	Jamuna	26	F	53	166	htn	39	1.7	107	39.49	3	1100	8.92	1.12	10.7	8.34	IgA Nephropathy	3	1
30	Manikandan	39	M	60	158	htn	51	1.9	110	44.3	3	650	3.319	1.99	4.97	5.84	IgA Nephropathy	3	1
31	Vishvanathan	35	M	66	175		25	0.8	113	120.3	1	2450	2.57	0.58	3.61	3.72	IgA Nephropathy	3	1
32	Bhagyarajan	35	M	60	177	htn,drug	24	0.8	93	109.4	1	1250	8.89	1.44	12.3	10.3	Membranous	3	1
33	Devika	32	F	51	156	htn,sle	110	5.2	96	11.77	5	1800	2.06	0.6	3.45	3.73	not done	3	2
34	Susaimary	32	F	67	164		32	1.3	94	61.85	3	1150	7.52	0.92	10	9.72	DPGN	3	1
35	Dilli Babu	16	M	48	167	htn	98	4.5	115	18.37	4	650	2.46	2	5.06	4.08	FSGS	3	1
36	Seetalakshmi	51	F	64	161	nsaid	23	1	114	63.29	2	2350	8.52	0.44	10	9.81	Membranous	3	1
37	Abhirami	31	F	50	168	htn,sle	61	3.1	87	19.53	4	650	5.78	1.68	9.21	7.13	Membranous	3	1
38	Sangeeta	14	F	38	154		28	0.9	97	59.11	2	1700	2.691	0.62	3.9	3.78	MPGN	3	1
39	Sundari	58	F	67	165	htn,nsaid	56	2.5	112	24.42	4	950	7.53	1.37	10.3	9.56	Membranous	3	1
40	Selvi	21	F	55	159	htn	54	1.3	160	55.94	2	1250	7.41	0.79	8.9	8.86	Membranous	3	2
41	Mariagracy	29	F	48	163	hypothyroid	35	1.2	91	49.33	2	2700	3.62	0.42	4.68	5.31	IgA Nephropathy	3	1
42	Jayaprakasam	28	M	49	171		42	0.8	103	95.28	1	1250	4.89	1.1	11.3	9.95	Membranous	3	1
43	John Basha	55	M	73	161	hcv,nsaid	21	0.9	92	95.76	2	1300	4.33	1.02	6.9	8.13	DPGN	3	1
44	Selvi	25	F	45	155	htn	69	2.4	73	23.96	4	750	3.416	1.04	8.73	6.81	IgA Nephropathy	3	1
45	Sangeeta	14	F	41	156		24	1.2	90	47.83	2	950	6.11	0.74	9.85	6.93	MPGN	3	1
46	Mohan	51	M	59	163	htn,nsaid	23	0.9	82	81.03	2	1650	4.35	0.69	5.37	6.12	FSGS	3	1
47	Arunkumar	36	M	71	159		29	1.2	87	85.46	2	2200	3.48	0.63	3.34	4.63	RPGN	3	1
48	Manikandan	42	M	77	169	htn,nsaid	47	1.7	114	61.65	3	2400	6.26	0.59	5.74	6.72	FSGS	3	1
49	Senthilkumar	42	M	76	159	htn,nsaid	33	2.5	174	41.38	3	1650	7.36	0.64	10.2	9.15	Membranous	3	1
50	Sathyamoorthy	47	M	71	175	htn,nsaid	43	2.4	79	38.21	3	1250	4.24	1.18	5.77	7.02	FSGS	3	1

51	Kalaivani	48	F	62	161	nsaid	59	2.9	73	21.85	4	1350	4.64	0.86	3.55	4.13	IgA Nephropathy	3	1
52	Subburajan	22	M	44	171		44	1.7	134	42.42	2	1350	6.57	1.11	4.55	6.88	DPGN	3	1
53	Manoharan	47	M	72	159	htn,nsaid	66	2.1	91	44.29	3	2150	2.54	0.68	3.99	5.66	not done	3	1
54	Joseph	67	M	59	174	htn,nsaid	41	1.6	114	37.39	3	1450	4.23	0.99	4.97	6.75	not done	3	1
55	Sekar	56	M	57	172	htn,nsaid	41	1.2	116	55.42	2	2100	5.21	0.68	7.52	5.74	CGN	3	2
56	chandrashekhar	55	M	61	160	hbv,nsaid	24	0.8	69	90.02	2	2500	2.774	0.46	3.14	3.62	Myeloma	1	1
57	Kavitha	67	F	65	154	htn,hypothroid,drug	24	0.9	94	58.58	3	1400	4.15	0.75	6.47	5.65	RPGN	3	1
58	Md. Ali	48	M	69	176	htn,nsaid	52	2.2	144	40.08	3	1450	4.77	1.21	7.45	6.75	CGN	3	1
59	Poongodi	17	F	47	162	htn	46	2	97	32.12	3	1150	2.88	1.01	3.24	3.77	IgA Nephropathy	3	1
60	Kannan	15	M	42	151	htn	42	2.7	84	27.01	3	550	7.47	2.18	4.55	5.41	IgA Nephropathy	3	1
61	Hari	30	M	64	166	htn	112	4.5	120	21.73	4	2450	2.34	0.49	6.89	8.43	not done	3	2
62	Arulselvi	24	F	49	157	sle	17	0.8	73	78.94	1	1450	4.52	0.66	5.89	5.25	DPGN	3	1
63	Revathi	25	F	62	164	htn,sle,drug	87	2.3	102	34.44	3	1400	4.42	0.79	6.72	6.7	Membranous	3	2
64	Ambika	15	F	52	155	htn	55	4.2	91	17.2	4	700	5.6	1.02	11.2	6.6	DPGN	3	1
65	Narassaiah	62	F	61	160	htn,nsaid	112	6.2	94	8.527	5	550	4.5	1.46	5.2	4.1	not done	3	1
66	Aadi	17	F	43	153	htn	63	3.3	76	17.81	4	1050	2.6	0.76	6.01	4.8	RPGN	3	1
67	Durai	52	M	74	177	nsaid	63	1.3	111	69.57	2	1300	6.63	1.03	7.76	9.44	CGN	3	1
68	Murugesan	44	M	71	175	nsaid,drug	28	0.9	85	105.2	1	1150	3.79	1.25	5.97	3.9	IgA Nephropathy	3	1
69	Munusamy	33	M	59	166		14	0.8	86	109.6	1	1700	4.6	0.76	4.25	5.5	CGN	3	1
70	Nataraj	19	M	57	164		42	1.4	82	68.42	2	1850	3.7	0.84	3.11	3.8	RPGN	3	1
71	Sarvanan	29	M	63	162	htn	24	1.1	136	88.3	2	2400	3.1	0.65	3.22	4.32	IgA Nephropathy	3	1
72	Venkatachalam	74	M	76	171	htn,hbv,nsaid	67	3.7	114	18.83	4	2650	2.6	0.62	4.89	6.4	DPGN	3	1

Wt - Weight of patient in Kg.; Ht - Height of the patient in cms.;S. Cr - serum Creatinine ; RBS - Random Blood Sugar ; eGFR - estimated Glomerular Filteration Rate using the Cockcroft-Gault equation;CKD - Stage of CKD based on Estimated Glomerular Filteration Rate ; 24 hr Vol - the volume of urine collected over 24 hours measured in ml.;ACR - early morning spot urine Albumin Creatinine Ratio;ur cr conc - measured urinary Creatinine Concentration expressed in mg/ml.;PCR - early morning spot urineProtein Creatinine Ratio; 24 Hrs U Pr - the 24 hours urinary protein excretion expressed in grams. Dipstic - result of use of Dipstick test (For Eg: 3 = +++ Protein),attmpt – number of attempts done for adequate 24 hours urine collection; FSGS – Focal Segmental Glomerular sclerosis; CGN – chronic Glomerulo sclerosis; MPGN – Membrano-Proliferative Glomerulo-Nephritis ;DPGN – Diffuse Proliferative Glomerulo Nephritis ; membranous – Membranous Nephropathy ;myeloma –Renal affection due to Myeloma(Plasma Cell Tumor); htn- systemic Hypertension ;hbv- Chronic Infection with Hepatitis B Virus;hcv- Chronic Infectin with Hepatitis C virus;drug- use of native medicine , or drugs known to cause renal injury except for NSAID ;nsaid- Non – steroidal Anti Inflammatory Drug use ;sle- Systemic Lupus Erythematosus ; hiv – Infection with HIV virus ;